

Pattern of breathing and lung receptor activity in an animal model of pulmonary emphysema

by

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DECLARATION

I declare that this thesis entitled "Pattern of breathing and lung receptor activity in an animal model of pulmonary emphysema" submitted for the degree of Doctor of Philosophy at the University of Edinburgh, is composed by myself and is the result of my own work except where stated in the text.

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1997

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ABSTRACT

This thesis investigates the pattern of breathing, lung reflex responses and activity of vagal lung receptors in an animal model of emphysema. The specific aim was to investigate if changes in pulmonary receptor activity are produced in an animal model of emphysema, and if these can be related to any changes in breathing pattern and lung reflex responses occurring in the diseased model. Emphysema was induced in rats by an endotracheal instillation of the proteolytic enzyme papain (120 mg/kg body weight). In control and emphysematous anaesthetised rats the pattern of breathing during eupnoea and during administration of 4% and 6% CO₂ was measured. The reflex responses to lung inflation and deflation pressures of 5 and 10cm were also recorded. Activity was recorded from either slowly adapting, (SARs) or rapidly adapting receptors, (RARs) in single fibres of the left vagus nerve during the above conditions. After bilateral vagotomy the pattern of breathing was measured in both groups of rats. The alveolar walls of the papain treated rats had a significantly increased mean linear intercept value of 109.3 \pm 2.7 mm compared to 81.5 \pm 3.1 mm in the control rats, ($P<0.01$), indicating that emphysema had been induced. Breathing frequency in the diseased rats with the vagi intact was slightly slower than the controls. Tidal volume was similar in both groups of rats 2.85 \pm 0.2 ml in the emphysematous and 2.83 \pm 0.2 ml in the controls. Breathing 4% and 6% CO₂ increased tidal volume in both groups of rats although this response was more vigorous in the controls, breathing frequency was not significantly altered in either group. Without the influence of the vagi the diseased rats breathed slightly faster than the controls. The Hering-Breuer inflation reflex response to both 5 and 10cm.H₂O pressure was significantly longer in the emphysematous rats. The Hering-Breuer apnoea on lung inflation was 2.7 \pm 0.48s and 6.3 \pm 1.4s in the control and emphysematous rats respectively at 5cm.H₂O pressure, ($P<0.05$) and 17.3 \pm 1.9s in the controls and 27.8 \pm 2.8s in the emphysematous rats at 10cm.H₂O pressure, ($P<0.01$). The deflation reflex was similar in both groups at both 5 and 10cm.H₂O pressure. The proportion of SARs to RARs was the same in both groups. Sixty-nine percent of the receptors were SARs in controls and 68% in the diseased group. The SARs from both groups of rats could be subdivided into those showing activity only during inspiration, those having activity in inspiration and at the beginning of expiration and those displaying activity throughout the respiratory cycle. Activity of the receptors was measured in terms of peak, mean and minimum firing frequency per

respiratory phase. The SARs of the emphysematous rats were significantly more active than those of the control rats during eupnoeic breathing. The peak frequency of SARs was 86.1 ± 1.98 Hz in the controls and 91.2 ± 1.98 Hz in the diseased rats, ($P < 0.05$). In eupnoea the RARs of the emphysematous rats were significantly more active than those of the controls. The RARs had a peak firing frequency of 87.5 ± 6.10 Hz in the controls and 118.8 ± 5.71 Hz in the emphysematous, ($P < 0.001$). The mean firing frequency of the RARs during eupnoea was 28.77 ± 1.25 Hz in the controls and 34.58 ± 1.25 Hz, ($P < 0.01$). The SARs of the emphysematous rats had a greater firing rate and statistically significant slower adaptation response on lung inflation than the control rats. The SARs from the control rats adapted by 60.8 ± 3.3 % and 37.7 ± 2.6 % at 5cm and 10cm.H₂O pressure in the first 0.75s following inflation respectively while SARs from the diseased rats adapted by only 49.6 ± 2.2 and 31.1 ± 2.2 % at 5 and 10cm.H₂O pressure respectively. During lung deflation RARs of the diseased rats had a slightly greater activity than those of the controls. RARs had a mean frequency of 62.7 ± 6.9 Hz in the controls and 71.2 ± 6.1 Hz in the diseased rats in the 1st breath of deflation to -5cm.H₂O, although this difference was not statistically significant.

SAR activity increased in both normal and diseased rats in response to the rats breathing 4 and 6% CO₂. The mean frequency of the SARs increased from 57.4 ± 1.98 Hz in the control rats in eupnoea to 67.4 ± 3.0 Hz at 4% CO₂ and to 68.3 ± 2.9 Hz at 6% CO₂. In the emphysematous rats, activity of the SARs increased from 58.3 ± 1.33 Hz during eupnoea to 62.0 ± 1.6 Hz at 4% CO₂ and to 65.8 ± 1.9 Hz at 6% CO₂. When breathing 4 and 6% CO₂ RAR activity decreased in both groups of rats. The RARs mean firing frequency during eupnoea of 28.77 ± 1.25 Hz in the controls fell to 19.45 ± 1.5 Hz and 19.68 ± 1.4 Hz when the rats breathed 4 and 6% CO₂ respectively, while in the emphysematous rats the activity of the RARs fell from 34.58 ± 1.25 Hz during eupnoea to 27.34 ± 1.5 Hz at 4% CO₂ and to 24.54 ± 1.3 Hz at 6% CO₂.

The slower pattern of breathing in the intact state and the stronger Hering-Breuer inflation reflex of the diseased rats can be accounted for by the increased activity of the SARs in these rats. The changed receptor activity from both SARs and RARs recorded from in this animal model of emphysema prompts the suggestion that these receptors might contribute to changes in respiratory drive, the inefficient breathing patterns and dyspnoea seen in human emphysema.

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LIST OF ABBREVIATIONS

Cst	Static compliance
FRC	Functional residual capacity
L_m	Mean linear intercept
$PaCO_2$	Arterial carbon dioxide tension
PaO_2	Arterial oxygen content
Pst	Elastic recoil pressure of the lungs
Te	Time taken for expiration to occur
Ti	Time taken for inspiration to occur
TLC	Total lung capacity
TTL	Transistor transistor logic
\dot{V}	Respiratory flow rate
Vt	Tidal volume

CHAPTER 1

INTRODUCTION

THE DISEASE EMPHYSEMA.

Emphysema is a major public health problem with millions of sufferers world wide. Emphysema causes suffering, distress, greatly reduces the quality of life and is ultimately the cause of death in many people with the condition. In emphysema destruction of lung connective tissue reduces the tethering of the airways of the pulmonary interstitium, leads to premature collapse of the airways, and increases the unevenness of the distribution of the inspired air to different regions of the lungs. All these changes hamper the transfer of carbon dioxide and oxygen between the blood and the alveolar air.

HISTORY OF THE DISEASE.

The symptoms of emphysema and other chronic respiratory diseases involving cough, laboured breathing and breathlessness have been known since ancient times. However the specific clinical features of emphysema could not be established until the anatomic pathology of the disease was recognised. The first clear anatomic description of emphysema was provided by Laennec in 1819. Using air dried, inflation fixed lung specimens he distinguished between vesicular emphysema in which alveoli were enlarged and interstitial emphysema in which air-containing spaces were found in the interstitium. Little was added to Laennec's anatomical descriptions of emphysema for over 100 years. Microscopic description of emphysema was produced in 1848 by Rainey, (Snider, 1992). Emphysema was thought to result from over distension of alveoli with rupture of the walls due to a one way valve type of bronchial obstruction produced by catarrh. This explanation proved to be inaccurate for generalised emphysema. Post World War II it was recognised that localised forms of emphysema resulted from inflammation, but no cause for the general condition was available. Subsequently studies into emphysema began to focus their attention on the connective

tissue of the lung; but it was not until the discovery of an inherited deficiency of α_1 anti-trypsin in 1963 and the experimental production of emphysema in animals by a single intratracheal injection of the elastolytic enzyme papain that the importance of proteolysis was recognised.

The presence of emphysema at autopsy and the clinical syndrome of chronic bronchitis were recognised long before cigarette smoking became a widespread form of tobacco use. However the disease became more common as tobacco use increased.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

In the 1950s it was realised that the term chronic bronchitis was used by British physicians to describe the same condition that physicians in the United States called emphysema. The use of these terms emphysema and chronic bronchitis have been generally superseded by the term chronic obstructive pulmonary disease (COPD). COPD includes the conditions of chronic bronchitis, emphysema, bronchiolitis, bronchiectasis, asthma and the now rare condition of rheumatoid bronchiolitis.

Due to confusion in usage of the terms "chronic bronchitis" and "emphysema" strict definitions of the diseases were necessary. Chronic bronchitis was defined as chronic production of sputum. Chronic being defined as occurring on most days for 3 or more months in the year, for 2 or more successive years, (Ciba Guest Symposium Report, 1959). Also the excess sputum produced should not be due to specific diseases such as tuberculosis or bronchiectasis. In 1962 the American Thoracic Society produced a strict definition of emphysema defining it as "a permanent, abnormal enlargement of any part of the gas-exchanging structures of the lung, accompanied by destruction of the respiratory tissue", (American Thoracic Society, 1962).

DEFINITION AND DESCRIPTION OF EMPHYSEMA.

Pulmonary emphysema has since been defined "as a condition of the lung characterised by abnormal permanent enlargement of the airspaces distal to the terminal bronchioles accompanied by destruction of their walls but without any obvious fibrosis", (Snider, Kleinerman, Thurlbeck and Bengali, 1985). This most recent definition of emphysema produced at the National Heart, Lung and Blood Institute, (N.I.H.) workshop, clarified the meaning of destruction in emphysema. The N.I.H. defined destruction in emphysema as "non-uniformity in the pattern of respiratory airspace enlargement, so that the orderly appearance of the acinus and its

components is disturbed and maybe lost", (Snider, Lucey and Stone, 1986). The National Heart, Lung and Blood Institute, (N.I.H.), Definition of Emphysema Workshop stated that fibrosis was not usually an evident part of emphysema, (Snider, Kleinerman, Thurlbeck and Bengali, 1985). Diseases of the lung which have airspace enlargement accompanied by fibrosis were excluded by their definition of emphysema since clinical, radiologic, and functional features of patients with such diseases are different from those found in the usual patients with emphysema. The N.I.H. committee concluded that airspace enlargement associated with fibrosis should be excluded. By this definition Emphysema has become a subset of pathological respiratory airspace enlargement, defined as "an increase in airspace size as compared with the airspaces of normal lungs", (Snider, Kleinerman, Thurlbeck and Bengali, 1985).

Respiratory airspace enlargement can be classified into three main types, (Thurlbeck, 1991):

1.) Simple Airspace Enlargement.

This can be of a congenital nature as in Congenital Lobar Overinflation and Down's syndrome, or may be acquired due to secondary loss of lung volume or acquired loss due to ageing.

2.) Emphysema.

There are several types of emphysema: Proximal acinar (centriacinar) emphysema, Panacinar emphysema, Distal acinar emphysema and Bullae.

3.) Airspace Enlargement with Fibrosis.

Irregular airspace enlargement and a condition known as Honeycomb lung.

Since the proceedings of the N.I.H. workshop were published, workers at the Meakins-Christie laboratory in Montreal have described the "destructive index" (DI) of the disease as seen in histologic slides of emphysematous lung, (Saetta et al 1985). They have raised the question of whether a redefinition of emphysema should be considered. The DI has three components: 1.) obvious emphysema, 2.) abnormal breaks in alveolar walls, and 3.) fibrosis. The implications from this work is that DI might be a better way of defining and recognising emphysema than the N.I.H. definition, and that the recognition of abnormal breaks in the alveolar wall might be a more sensitive and objective assessment of the disease. These investigators also showed that the DI was related to pulmonary function loss and to smoking, (Saetta et al, 1985). Another fact of interest in this study is that the DI could be abnormal in smokers even when airspace size was within what they considered to be normal limits. Thus the criterion of airspace enlargement may become unnecessary in the definition

of emphysema, and destruction of the airspace wall may be the only criterion required, (Thurlbeck, 1991). Although studies have shown that the largest component of the DI is due to obvious emphysema, and also that DI does not relate any better to pulmonary function abnormalities than do naked eye assessments of emphysema, (Saito, Cagle, Berend, and Thurlbeck, 1989).

CLASSIFICATION OF EMPHYSEMA.

The severity of emphysema is more important than the type of emphysema, (Thurlbeck, 1991). However classification leads to a closer examination of the etiology and pathogenesis of emphysema as well as the clinicopathologic correlations.

CENTRIACINAR (PROXIMAL ACINAR) EMPHYSEMA.

In proximal acinar emphysema, the proximal part of the acinus - the respiratory bronchioles is dominantly or selectively involved. Two forms are recognised, (Thurlbeck, 1991).

1. Proximal Acinar Emphysema due to dust; (Simple Coal Pneumoconiosis, Simple Pneumoconiosis, Focal Emphysema).

This disease is found in coal miners and other people subjected to nonfibrogenic dusts, (Thurlbeck, 1991). It is characterised by a lot of pigment with generally only a slight increase in the size of the respiratory bronchioles.

2. Proximal Acinar, Non industrial Emphysema (Centrilobular Emphysema, CLE) This is the commonest form of emphysema associated with the clinical syndrome of emphysema, (Thurlbeck, 1991). It is found usually only in smokers and, like the dust related emphysema, the lesions involve the respiratory bronchioles. The lesions are mostly destructive in this type of emphysema compared to the dilatation of the bronchioles which dominates in the dust induced emphysema. Pigmentation is slight or absent, (Thurlbeck, 1991). Centrilobular emphysema mostly occurs in the upper lobe and superior segment of the lower lobe. There is usually evidence of inflammation and narrowing of the supplying bronchiole. The severity of centrilobular emphysema is very irregular exhibiting considerable variation from lobule to lobule and within a lobule itself. It is generally accepted that non industrial emphysema, which dominantly, but not solely involves the proximal acinus, should be referred to as CLE, (Thurlbeck, 1991).

PANACINAR (PANULOBULAR EMPHYSEMA) (PLE).

Panacinar emphysema is the term applied to the form of emphysema where the acinus is more or less uniformly enlarged and destroyed, (Thurlbeck, 1991).

ALPHA₁- ANTITRYPSIN (PROTEINASE INHIBITOR) DEFICIENCY AND FAMILIAL EMPHYSEMA.

The amount and type of proteinase inhibitor is determined by a pair of codominant alleles, (Thurlbeck, 1991). Pi^M is the normal phenotype and Pi^Z is associated with an increased prevalence of emphysema particularly in smokers. Symptomatic emphysema occurs in relatively young patients in their early forties or younger. Some individuals with a genetic deficiency for alpha₁ anti-trypsin can develop emphysema even if they are non-smokers. Smoking however can hasten the onset of emphysema in alpha₁ anti-trypsin deficient individuals, (Kueppers and Black, 1974).

Alpha₁ anti-trypsin deficiency is responsible for 2% of all cases of emphysema in the United States, (Gadek and Crystal, 1983). The emphysema associated with this disease is regarded as panacinar in type, (Pratt and Kilburn, 1970).

PANACINAR EMPHYSEMA ASSOCIATED WITH OLD AGE.

A small proportion of older non-smoking patients develop emphysema, (Thurlbeck, 1991). It is panacinar in type and is usually mild and found along the margins of the lower lobes. It is asymptomatic, (Thurlbeck, 1991).

BULLAE.

Bullae have been defined as a form of emphysema where airspaces within the parenchyma are more than 1cm in diameter in the distended state, (Ciba Guest Symposium Report, 1959). Such spaces are quite commonly seen radiologically but are much less common pathologically. Some airspaces that are visible radiologically actually contain lung parenchyma pathologically and are not true cystic spaces. There are three types of bullae, (Reid, 1967) two of which are associated with emphysema.

EPIDEMIOLOGY OF EMPHYSEMA.

The numerous surveys of the incidence of emphysema have been reviewed elsewhere, (Sobonya and Burrows, 1983), (Thurlbeck, 1976). Some considerable discrepancies between the different studies have been found, that are probably due to both inter-observer variation and true differences. The studies were almost invariably centred on industrial cities. The consensus was that two thirds of the males and about one fourth of the females sampled had emphysema, based on studies at autopsy, of subjects with an average age of about 60 years, (Thurlbeck, 1991). Emphysema is uncommon in people under 40 years of age. The percentage incidence of emphysema rises in frequency until the seventh decade, and then declines, (Thurlbeck, 1991). Smoking is accompanied by a dramatic increase in the occurrence of emphysema, (Thurlbeck, 1991). There are great international variations of the several different types of emphysema, Proximal acinar (centriacinar) emphysema, Panacinar emphysema, Distal acinar emphysema and Bullae which have already been described. To what extent preclinical emphysema is present in the general population is not known.

SYMPTOMS AND SIGNS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

Since chronic bronchitis and emphysema are both smoking related diseases they often occur together in patients who smoke. The characteristic symptoms of chronic bronchitis are cough, sputum, wheeze and breathlessness, (Crofton and Douglas, 1981). The onset of the illness is often dated by patients to some exacerbation of cough and sputum, which left them with a degree of disability that began to seriously interfere with daily life, (Crofton and Douglas, 1981). However the patients had usually suffered from smokers' cough for many years prior to this exacerbation, (Crofton and Douglas, 1981). A spectrum of patients with COPD are sometimes described, (Crofton and Douglas, 1981). Patients having symptoms due to a condition of pure emphysema are placed at one end of the spectrum and patients with symptoms due to pure chronic bronchitis are placed at the other end. At the 'pure emphysema' end of the COPD spectrum is a group of patients in whom cough, sputum and wheeze appear to be relatively unimportant and whose major disability is breathlessness, (Crofton and Douglas, 1981). The history is often of progressive severe dyspnoea, sometimes starting with some apparently mild respiratory infection. Within a short time the patient becomes a respiratory invalid and may die within a few years, usually from respiratory failure without cor pulmonale. Some of these emphysematous

patients are able to maintain their PO_2 by hyperventilation, and also keep the PCO_2 within normal limits up to a late stage, (Crofton and Douglas, 1981). These are the 'pink puffers' as opposed to the 'blue bloater' set of patients. In some of the patients their alveolar walls seem to 'vanish' steadily into emphysematous bullae, (Crofton and Douglas, 1981). This is more common in North American patients than in Britain, (Crofton and Douglas, 1981). Most of them, but not all, are cigarette smokers; the causal role of tobacco is much less certain than in classical chronic bronchitis and, due to the geographical distribution of the disease, atmospheric pollution is not thought to be important. A few cases are associated with α_1 -antitrypsin deficiency, (Crofton and Douglas, 1981), (see the section on etiology and pathogenesis of emphysema).

Much more common in Britain, both clinically and pathologically, is the patient who has evidence of both diffuse airway obstruction, partially reversible, and some emphysema, and in whom cough and sputum are prominent features, (Crofton and Douglas, 1981). In an autopsy study in Canada considerable emphysema was often present and associated cor pulmonale; indeed only 15% of patients with chronic airways obstruction were found not to have emphysema, (Thurlbeck et al 1970).

Such findings support the popular view that the extent of irreversible airways obstruction is mainly correlated with the extent of emphysema. With use of the *in vivo* CT scan it has been found that the clinicopathologic pattern of the blue and bloated patient (airways obstruction, hypoxaemia with CO_2 retention pulmonary hypertension, and a history of cor pulmonale) can exist in patients with varying degrees of anatomic emphysema. Other patients with the clinicopathologic pattern of the pink and puffing patient; (similar airway obstruction, but only modest hypoxaemia and a low or normal arterial PCO_2 , little pulmonary hypertension, and no cor pulmonale) can also show some, extensive or only a little emphysema. These results thus contradict the older notion that blue bloaters had mainly chronic bronchitis and the pink puffers mainly emphysema. However they confirm the earlier physiopathologic studies that found the degree of emphysema, as assessed by pathologic examination of both lungs post-mortem, did not relate to the pattern of pink puffer or blue bloater.

ETIOLOGY AND PATHOGENESIS OF EMPHYSEMA.

The "protease-antiprotease" theory of how emphysema is produced in the lungs, in its simplest form, is based on the premise that the structural proteins of the lung, notably collagen and elastin, are continually being synthesised and broken down. Two almost simultaneous studies in the sixties were initially responsible for this theory. In 1963 Laurell and Eriksson noted that patients with reduced α_1 -globulin

levels on serum electrophoresis had an increased frequency of premature emphysema, this being familial, (Laurell and Eriksson, 1963). Later, in 1965, Gross and colleagues went on to show that proteolytic enzyme derived from the papaya tree could produce emphysema when instilled into the trachea of experimental animals, (Gross, et al. 1965). Subsequent investigations, (Janoff, 1985) showed that elastase is a potent producer of experimental emphysema but collagenase is not. The α_1 -globulin defect was related to α_1 -trypsin deficiency, and α_1 -globulin had a significant antielastase activity. Smokers were found to have increased numbers of macrophages and polymorphonuclear leukocytes (PMNs) in bronchoalveolar lavage fluid. The macrophages were metabolically highly active and contained elastase and other proteolytic enzymes. Smokers were also shown to have reduced antiprotease activity, (Janoff, 1985) although this finding remains controversial, (Thurlbeck, 1991).

In its now expanded form, the protease-antiprotease theory states that the normal balance between proteolysis and antiproteolysis may be disturbed, and when proteolysis dominates emphysema ensues. This usually occurs in smokers, where it is proposed that cigarette smoke causes alveolar macrophages to cluster around the small terminal bronchioles of the lungs, (Flenley, 1986). A diagrammatic representation of the proteolytic theory of emphysema production in smokers can be seen in Figure 1-1. Cigarette smoke can stimulate alveolar macrophages to produce and or secrete chemoattractants, (Hunninghake, Gadek and Crystal, 1980). The alveolar macrophages secrete or produce chemoattractants and secretagogues such as leukotriene B4 (LTB4) and a peptide attractant for neutrophils, (Janoff, 1985). Neutrophils can also be attracted by complement factors and by components of cigarette smoke itself e.g. nicotine, (Totti, McCusker, Campbell, Griffen and Senior, 1984). These neutrophil attractants attract polymorphonuclear leucocytes to the lungs from within the circulation, (Flenley, Downing and Greening, 1986). The neutrophils are then believed to release their potent human neutrophil elastase (HNE), (Flenley, Downing and Greening, 1986), see Figure 1-1. The HNE released is a powerful proteolytic enzyme that has the capability of attacking collagen, elastin, proteoglycans and fibronectin. Some investigators have found that the macrophages from smokers secrete an elastase, (Rodriguez, White, Senior and Levine, 1977). Since elastase attacks collagen, elastin, proteoglycans, and fibronectin, which are the major macro-molecules of the lung interstitial matrix, destruction of the lung ensues. This enzyme, elastase, however also readily binds to a component of the plasma proteins, α_1 -proteinase inhibitor (α_1 -antitrypsin), which then forms an inactivated complex with HNE, so destroying the elastolytic activity of the enzyme, (Flenley, 1986).

In the majority of patients with emphysema, protease-antiprotease imbalance is not evident, (Janoff, 1985) but since most patients who develop emphysema are smokers it has been suggested that smoking increases the elastolytic activity in the lungs and/or depresses their antielastolytic protective mechanisms, (Janoff, 1985). Yet by no means all smokers develop clinically evident emphysema in their lifetimes, (Janoff, 1985). This suggests that clear and as yet unsolved problems remain in the protease-antiprotease theory of emphysema, although it may be an important part to the whole and real pathogenesis of emphysema in man.

Oxidants produced by smoke itself and by phagocytic cells in the lungs in response to smoke particles may be of importance in the pathogenesis of emphysema, (Hoidal, McCusker, Marshall and Rao, 1991). Antioxidant mechanisms in the lungs of cigarette smokers appear to be more activated, perhaps in response to greater oxidant stress, (Hoidal, McCusker, Marshall and Rao, 1991). Within cells, antioxidant enzymatic and non-enzymatic defences help prevent the accumulation of oxygen radicals and peroxides, (Hoidal, McCusker, Marshall and Rao, 1991). Intracellular superoxidase dismutases and catalase activities, which control O_2^- and hydrogen peroxide levels respectively are twice as high in the alveolar macrophages of cigarette smokers compared to non-smokers, (McCusker and Hoidal, 1990).

Oxidants may promote protease-mediated destruction of the lung matrix by either stimulating recruitment of phagocytes to the lung and thus increasing the protease burden, (Hoidal, McCusker, Marshall and Rao, 1991) and / or by oxidative inactivation of the α_1 -proteinase inhibitor, (Hoidal, McCusker, Marshall and Rao, 1991). Modification of lung matrix molecules by the action of oxidants may make them more susceptible to protease attack, (Hoidal, McCusker, Marshall and Rao, 1991). The concept that degradation of lung matrix molecules directly by oxidants may also be relevant as these agents are likely generated in large quantities in proximity to the structural proteins and basement membranes in the lungs of smokers, (Hoidal, McCusker, Marshall and Rao, 1991).

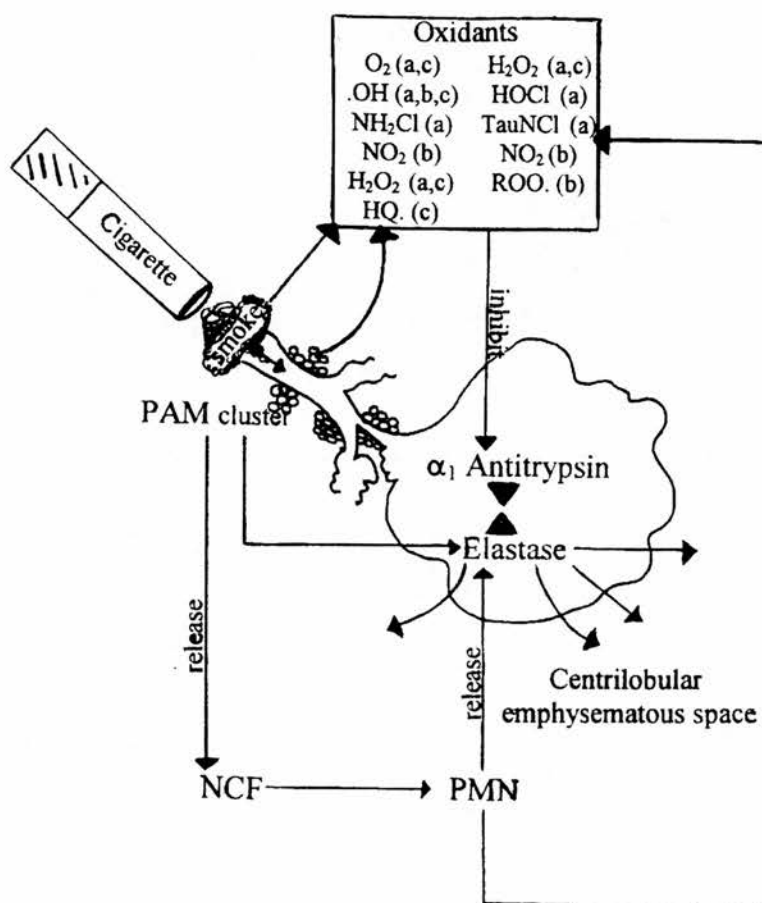


Figure 1-1: *Diagrammatic representation of the proteolytic theory of emphysema.*

Smoke from cigarettes causes alveolar macrophages (PAMs) to cluster around the airways. The PAMs release neutrophil chemotactic factor (NCF) that then attracts polymorphonuclear leucocytes (PMNs). These PMNs then release the proteolytic enzyme, human neutrophil elastase thought to be responsible for producing alveolar destruction.

DIAGNOSIS OF EMPHYSEMA.

A diagnosis of pulmonary emphysema must establish the presence of emphysema in the lung; where emphysema is defined as a condition of the lung characterised by abnormal permanent enlargement of the airspaces distal to the terminal bronchioles, accompanied by destruction of their walls, without any obvious fibrosis, (Snider, Kleinerman, Thurlbeck and Bengali, 1985). This definition clearly depends on the pathologic examination of the whole of both lungs, which have been fixed in inflation. The definition also requires that the range in size of normal distal airspaces be established, so as to determine what is abnormal.

PATHOLOGIC DIAGNOSIS.

Emphysema has been diagnosed microscopically by measuring the size of distal airspaces. These are estimated using the mean linear intercept (L_m) measurement of lung sections cut from paraffin-embedded blocks, taken from inflation fixed lungs, after correction for the shrinkage and distortion of such specimens from cutting, (Thurlbeck, 1967). The size of the distal airspaces are compared with normal values and thus a diagnosis of emphysema can be made. The mean linear intercept is the average distance between the intersections of alveolar walls on a line of known length randomly placed on a random lung section, (Thurlbeck, 1967). Alternatively the degree of emphysema has been assessed macroscopically by comparing the naked eye appearance of a cut slice with that seen in a standard picture (picture grading), (Thurlbeck, Dunhill, Hartung, et al 1970).

CLINICAL DIAGNOSIS.

Emphysema has traditionally been difficult to diagnose accurately in life and almost impossible to quantify, (Gould, Macnee, McLean, Warren, Redpath, Best, Lamb, and Flenley, 1988). Hyperinflation of the chest as assessed by clinical examination is often associated with emphysema, although hyperinflation may also occur in an acute attack of asthma and in the chronic asthmatic patient.

RADIOLOGIC DIAGNOSIS.

Chest radiograph remains unreliable for the exclusion of emphysema; however if emphysema is diagnosed from the radiograph, this will probably be found to be present at autopsy, (Flenley, 1991).

COMPUTER TOMOGRAPHY DIAGNOSIS.

The CT scan presents a visual display, conventionally as a grey scale, which depends on the electron density of the tissue contained within the pixel (or unit) of the display. In a display of the transthoracic CT scan since air has a lower electron density than that of lung tissue an emphysematous space that occupies several pixels will show up as a black area. In contrast the lighter areas represent tissues with a higher density, such as in the extreme case of bone in the vertebrae and the rib cage, (Flenley, 1991). Using a window setting on the CT scan close to that of lung tissue, emphysematous spaces can be distinguished from lung parenchyma visually, (Goddard, Nicolson, Laszlo, et al. 1982). The density of the airspace walls is close to that of water whereas the density of the alveolar gas in the enlarged distal airspaces is close to that of air. The visual distinction produced by the CT scan is however derived from numerical information that represents the radioabsorbance of each pixel, which is expressed as either a Hounsfield unit or EMI number, (Flenley, 1991). Thus quantitative differences in lung density can be related to the presence of emphysema. It appears that the quantitative CT scan, by direct comparison with morphometric measurements in the same resected lobe or lung, can accurately diagnose, locate and quantify emphysema by a noninvasive method in the lungs of living humans, (Gould, Macnee, McLean, Warren, Redpath, Best, Lamb, and Flenley, 1988).

RESPIRATORY FUNCTION IN EMPHYSEMA.

Hyperinflation can be measured by lung volumes as an increase in residual volume to total lung capacity ratio (RV/TLC). Low FEV_1 /FVC ratio and a low peak expiratory flow rate (PEFR) both indicate airway obstruction. When these are combined with a high RV/TLC ratio, they together form the classic obstructive pattern in respiratory function, which relates to the airflow obstruction but not necessarily to the emphysema of chronic obstructive lung disease. However, in patients with persistent air flow obstruction, as shown by persistently low FEV_1 or PEF measurements when made several times per day over many days, if not months, some studies have shown that FEV_1 does correlate with the degree of emphysema, (Thurlbeck, Henderson, Fraser, Bates, 1970).

NEURAL CONTROL OF BREATHING.

The present study investigates the effects that changes in the lung, produced by emphysema, have on activity from pulmonary mechanoreceptors which modify the process of breathing. It is therefore appropriate to consider briefly the effect of the receptors.

CENTRAL CONTROL OF BREATHING.

Respiration is a reflex activity that can be modified voluntarily, (Euler, 1986), (Long and Duffin, 1986). Regions in the brain and spinal cord control breathing and, generate, form and shape the respiratory pattern, (Euler, 1986). The respiratory information from these regions are integrated with each other in a hierarchical manner with some regions exerting more control over respiration than others, (Long and Duffin, 1986). This hierarchical control allows respiratory movements to be co-ordinated and integrated with complex behavioural acts, providing a system that allows breathing to take place while moving, speaking and eating, (Campbell and Howell, 1963). The activity of this 'central pattern generator' is modified by the activity of the lung mechanoreceptors studied in this thesis, (Coleridge and Coleridge, 1986).

Although complex control of breathing will provide an appropriate respiratory pattern, depending on other activities and requirements of the animal, a stereotypical but nearly normal breathing pattern can be maintained when the pons and medulla are separated from the rest of the brain, (Long and Duffin, 1986).

RESPIRATORY NEURONES.

Three major groups of neurones in the pons and medulla are concerned with breathing and produce the 'basic' breathing pattern, (Euler, 1986), (Long and Duffin, 1986). The pontine respiratory group of neurones is located in the area on the pons known as the 'pneumotaxic centre'. The other two groups of neurones are located in the medulla, these are the dorsal respiratory group and the ventral respiratory group which together form the 'medullary centre'.

Pneumotaxic centre.

The Pneumotaxic centre or (PRG) is the respiratory centre located in the rostral portion of the pons. It was termed the pneumotaxic centre as focal destruction in anaesthetised or decerebrate vagotomised animals results in apnoea (prolonged inspirations), (Mitchell and Berger, 1981). It is now more often referred to as the pontine respiratory group (PRG). Focal destruction studies have located the PRG within the nucleus parabrachialis medialis, the Kolliker-Fuse nucleus, and portions of the brachium conjunctivum, (Mitchell and Berger, 1981).

The pneumotaxic centre is also known as the pontine respiratory group (PRG). The PRG has three different types of units, ones which discharge solely during inspiration and the other only during expiration. In addition to units whose discharge is locked to inspiration or expiration, the pontine respiratory group also contains units which fire in a phase spanning manner, (Mitchell and Berger, 1981). The inspiratory modulated cells are concentrated in the ventral and lateral portions of the area described as the pneumotaxic centre, phase spanning neurones in the central core and expiratory neurones are located medially. This group of neurones or centre seems to be primarily concerned with adjustments in the duration of the phases of breathing and the rate of respiration, (Long and Duffin 1986). Electrical stimulation of this region causes premature switching of the respiratory phases, (Mitchell and Berger, 1981).

Medullary centre.

The medulla is probably the site of the dominant respiratory oscillator and is where visceral afferent fibres from receptors in the lungs, airways, and fibres from chemo- and baroreceptors synapse, (Mitchell and Berger, 1981). Information arising from receptors in the tracheobronchial tree and lung parenchyma travel in the Vagus nerve to the respiratory neurones in the medulla, see Figure 1-2.

Within the medullary centre there are two compact groups of neurones - the dorsal respiratory group, (DRG), and the ventral respiratory group, (VRG), (Mitchell and Berger, 1981). The DRG is located in the dorsal medulla close to the obex where there is a concentration of inspiratory neurones, though some expiratory neurones have been found here. The concentration is associated with the ventrolateral portion of the nucleus of the tractus solitarius (solitary tract), (Mitchell and Berger, 1981). In the ventral medulla the ventral respiratory group (VRG) is located in the nucleus ambiguus (NA) rostrally and the nucleus retroambiguus (NRA) caudally, respiratory neurones of the VRG are also located in the nucleus parambiguus, ambiguus and in the nucleus retrofacialis, (Mitchell and Berger, 1981). The neurones constituting the

VRG are a collection of both inspiratory and expiratory cells, (Mitchell and Berger, 1981).

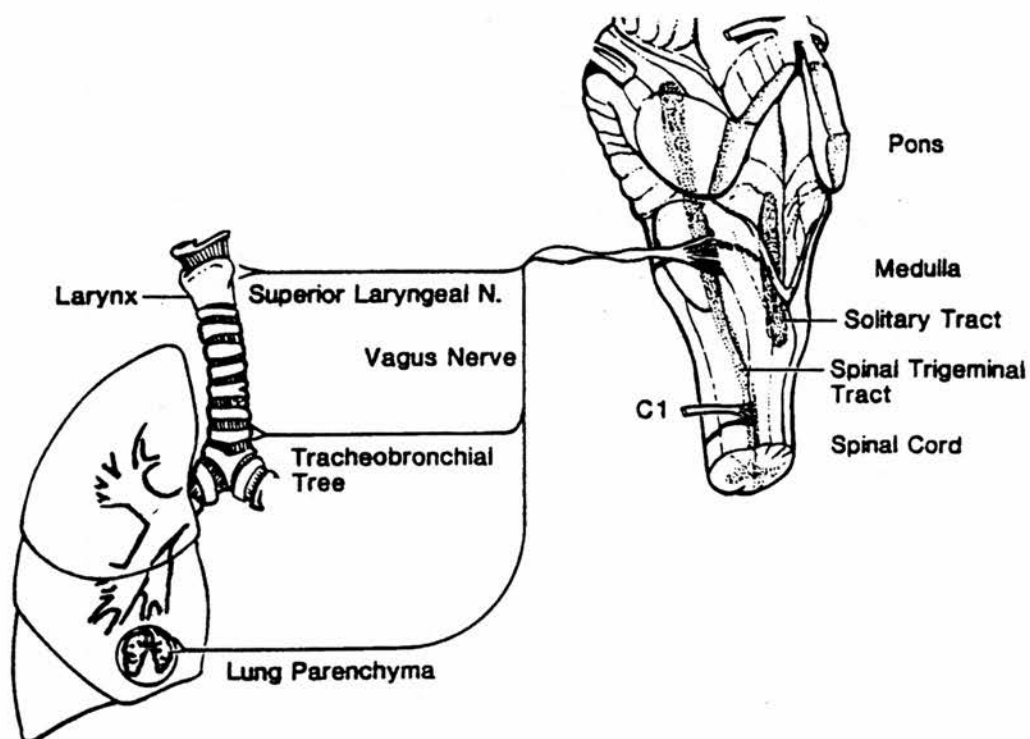


Figure 1-2: Afferent innervations of the airways and lung parenchyma showing their projections to the pons and medulla.

Dorsal respiratory group.

The DRG has been confirmed, by numerous studies in the cat, to be contained in a region extending from about 1 mm caudal to 2 mm rostral to the obex and just ventrolateral to the nucleus of the solitary tract, (Mitchell and Berger, 1981). The association of respiratory neurones with this region of the brain stem is important since the tractus solitarius is the afferent fibre tract for visceral afferents in IX and X cranial nerves, (Mitchell and Berger, 1981), see Figure 1:2. This suggests that primitive respiratory reflexes, involving activation of visceral afferents carried by these nerves, may involve neuronal connections between these afferents and the DRG. Similar compact formations of cells at this site have been reported in other animals and man, (Mitchell and Berger, 1981).

There are two types of inspiratory units in the DRG, $I\alpha$ and $I\beta$ cells, both types having an inspiratory rhythm of central origin, (Mitchell and Berger, 1981). $I\alpha$ cells are inhibited by lung inflation sufficient to inhibit phrenic activity whereas $I\beta$ cells are excited with lung inflation during inspiration and also activated by lung inflation applied during the expiratory pause, (Mitchell and Berger, 1981). $I\beta$ cells are thought to be excited by pulmonary afferents which inhibit inspiration; that they act as interneurons inhibiting $I\alpha$ cells; and their inspiratory activity is derived from excitatory synaptic connections from $I\alpha$ cells, (Mitchell and Berger, 1981). Lung inflation induced termination of inspiration does not alter the rate of rise of activity of the $I\alpha$ neurones but there is a markedly increased rate of rise in activity of the $I\beta$ cells, (Mitchell and Berger, 1981). $I\beta$ cells may play an important role both in the lung inflation induced termination of inspiration and in Head's paradoxical reflex, (Mitchell and Berger, 1981), (see section on reflexes). When lung inflation is applied during expiration the $I\beta$ units are only weakly excited and their sensitivity does not correspond to the observed expiratory phase prolongation, (Mitchell and Berger, 1981).

Within the DRG there is a group of cells, which are termed the P cells, that do not have a central inspiratory rhythm, nor projections to the spinal cord, but are extremely sensitive to lung inflation, (Mitchell, and Berger, 1981). These P cells are the suggested interneurons of the Hering-Breuer expiratory facilitatory reflex, but their role in inspiratory cut off is unknown. P cells may be a general interneurone, mediating airway reflexes that produce expiratory phase facilitation, (Mitchell and Berger, 1981).

Many visceral afferent fibres are thought to synapse directly on to neurones at this site as already discussed and probably the DRG neurones project to the PC and VRG, (Mitchell and Berger, 1981). In addition to these projections, most $I\alpha$ and $I\beta$

neurones project to the contralateral spinal cord. The DRG neurones may also directly excite phrenic motoneurones. The DRG appears to have a primary role in viscerosensory respiratory motor acts. In addition to respiratory phase switching, perhaps the site of primary respiratory pattern generation may reside in the DRG, (Mitchell and Berger, 1981).

The Ventral respiratory group.

The ventral respiratory group VRG in the ventral medulla consists of four neurones in the nucleus retroambiguus, parambiguus, and ambiguus, and in the nucleus retrofacialis. The neurones that make up the VRG each contain both inspiratory and expiratory cells, (Euler, 1986). The inspiratory and expiratory cells of the nucleus ambiguus differ in firing pattern, membrane potential change, and reflex responses from those of the nucleus retroambiguus, (Mitchell and Berger, 1981).

Nucleus ambiguus.

The NA contains cranial nerve respiratory motoneurones, whose axons are primarily in the recurrent laryngeal nerves. These axons innervate the muscles of the larynx where the abductor muscles are active during inspiration and the adductor muscles are active during expiration, (Mitchell and Berger, 1981). Expiratory neurones in the NA exhibit a decreasing firing frequency as the expiratory phase progresses, whereas the discharge pattern of the inspiratory neurones shows an increasing firing frequency as inspiration progresses, (Mitchell and Berger, 1981). Expiratory neurones in the NA appear to have a paradoxical response to CO_2 ; silence at high CO_2 and tonic activity at low CO_2 . It has been suggested that this may be important in CO_2 regulation, (Mitchell and Berger, 1981). During expiration laryngeal adductor muscle contraction results in glottal closure, which would be undesirable at conditions of high CO_2 . Thus the silence of expiratory NA neurones at conditions of high CO_2 and consequent lack of adductor contraction permits increased gas movement under these conditions, (Mitchell and Berger, 1981).

Moderate lung inflations during expiration activate the Hering - Breuer expiratory facilitatory reflex. NA expiratory neurones are paradoxically inhibited by lung inflations, (Mitchell and Berger, 1981).

Nucleus retroambiguus.

The NRA seems to have a definite organisation in the rostral-caudal direction. The inspiratory neurones are concentrated rostrally and the expiratory neurones caudally, (Mitchell and Berger, 1981). NRA expiratory neurones display a different firing pattern from those in the NA. They commence firing at the start of expiration; their frequency slowly increases, being highest late in the expiratory phase, (Mitchell and Berger, 1981).

INSPIRATORY AND EXPIRATORY FIRING NEURONES.

The NRA contains two types of inspiratory neurones which can be distinguished on the basis of firing pattern and projections, (Mitchell and Berger, 1981). One type, termed the late-inspiratory cell, has its peak firing rate late in the inspiratory phase. The other type, termed the early-burst cell, has its peak firing rate early in inspiration with a subsequent decline in activity, (Mitchell and Berger, 1981). Late-inspiratory neurones show a ramp-type depolarisation during inspiration; early-burst cells are rapidly depolarized at the start of inspiration, (Mitchell and Berger, 1981).

Expiratory neurones in the NRA are excited by lung inflation and almost all inspiratory neurones projecting to the cord show inhibition with lung inflation, (Mitchell and Berger, 1981). The NRA neurones provide the excitatory drive to expiratory intercostal and abdominal motoneurones, (Mitchell and Berger, 1981).

The VRG is a heterogeneous collection of neurones having a variety of functions. The respiratory neurones of the NA are primarily cranial motoneurones going to various accessory muscles of respiration, with the majority of these neurones innervating the laryngeal musculature. They receive orthodromic drive from the DRG, (Mitchell and Berger, 1981).

The three groups of neurones of the pontine respiratory group, the dorsal respiratory group and the ventral respiratory group are heavily interconnected and receive multiple respiratory inputs and afferent signals from the cardiovascular, somatic, and visceral afferent systems, (Cherniack, 1991).

The DRG and VRG contain premotor neurones that actuate phrenic and intercostal motor units in the spinal cord. Respiration is then further modified in the spinal cord by segmental reflexes from muscle and joint proprioceptors, however signals from these proprioceptors also ascend to the medulla and even to the cortex, (Cherniack 1991).

The action of spinal, pontine, and medullary respiratory groups can be substantially modified by projections from the cortex, hypothalamus and mid-brain.

These projections allow voluntary control of breathing as well as help co-ordinate the respiratory and vasomotor responses to exercise and stress, (Euler, 1986).

The respiratory cycle consists of three phases: inspiration, expiration and a postinspiratory interval in which both inspiratory and expiratory neurones are inhibited, (Cherniack, 1991). Inspiration consists of a ramp-like increase in the firing pattern of respiratory neurones that is 'switched off'. The activity of respiratory neurones is switched off, by the inhibitory signals conveyed over the vagus nerves from lung stretch receptors, by activation of expiratory neurones in the pons and medulla, or by a third type of neurone (postinspiratory) identified by Richter in the dorsal medulla that inhibits both inspiration and expiration, (Euler, 1986), (Long and Duffin, 1986) and (Richter, 1982). The inspiratory motor neurones begin to fire again after a pause, but in a declining manner, retarding the exit of air from the lung, which possibly improves gas exchange. Expiration follows this phase with air being pushed out of the body under normal conditions by the passive relaxation of the lungs. The expiratory muscles are electrically silent. When respiratory drive intensifies, the ramp increase in inspiratory activity grows steeper, inspiratory flow into the lungs accelerates, the duration of the postinspiratory phase decreases, and expiratory muscles, such as the abdominal muscles and the internal intercostal, contract more forcibly, (Euler, 1986). There is one expiratory muscle, the transverse sterni that is active in animals and probably in humans during quiet breathing, (Cherniack, 1991).

It is generally accepted that the oscillatory breathing sequence is the result of an interaction of a network of neurones (the central pattern generator), (Cherniack, 1991). This pattern of breathing being further modified by neurones firing with different time courses, (early and late inspiratory, early and late expiratory, and phase spanning neurones) in the DRG, VRG and PRG, (Long and Duffin, 1986). However the site of the central pattern generator has not yet been precisely determined. Also pacemaker cells have been identified from slice preparations of the medulla. They have been located both in ventral and dorsal positions but it is uncertain what role these pacemaker cells may have in normal breathing patterns in the intact medulla, (Long and Duffin, 1986), (Richter, 1982).

REFLEX AND BEHAVIOURAL CONTROL OF BREATHING.

REFLEXES.

Mechanoreceptors in the lung and chest wall can modify both the pattern and rate of breathing, (Coleridge and Coleridge, 1986), (Shannon, 1986). Sensory receptors in the lung communicate with respiratory neurones in the brain via the vagus

nerves, (Coleridge and Coleridge, 1986). There are three types of lung receptors: slowly adapting stretch receptors, rapidly adapting receptors also known as irritant receptors and C or J receptors, (Coleridge and Coleridge, 1986).

Respiratory reflexes evoked from receptors in the tracheobronchial tree and lung play a role in the control of breathing although the exact nature and extent of their controlling influence is not fully understood.

Respiratory reflexes have been extensively investigated since 1868 when Hering and Breuer first described the two respiratory reflexes which bear their names. They described a reflex respiratory occurrence evoked by inflation of the lungs. When the lungs of animals were inflated Hering and Breuer found a reflex decrease in frequency and force of inspiratory efforts, (the inflation reflex). They also found that deflation of the lungs evoked stronger and more frequent inspirations, (the deflation reflex), (Hering and Breuer, 1868). The ingenious work of Hering and Breuer was further extended by Head in 1889 who described a third reflex. He noted that when activity travelling from lung receptors was partially blocked, by cooling the vagus nerve, inflation of the lungs no longer inhibited inspiratory efforts, but caused a strong maintained contraction of the diaphragm, (Head, 1889). This reflex is known as Head's paradoxical reflex. All three reflex events are mediated by afferent nerve fibres in the vagus nerve.

Inflation reflex.

Adrian, in 1933, showed that the Hering-Breuer inflation reflex is mediated by slowly adapting pulmonary stretch receptors (SARs) and their vagal afferent nerve fibres, and that the reflex modifies the respiratory cycle and so changes the animal's pattern of breathing, (Adrian, 1933). After vagotomy the reflexes can no longer be evoked as sensory information from the lungs to the respiratory centre has been removed (Hering and Breuer, 1868). The altered pattern of breathing in the vagotomised state is attributed to this removal of tonic activity by receptors for this reflex, (Widdicombe, 1961). The inflation reflex is thought by some (Widdicombe, 1961) to adjust the rate and depth of breathing to be mechanically most economical, and that pulmonary stretch receptors act as sensing organs for the physical state of the lungs. Support for this idea comes from the fact that the volume/response characteristics in cats are changed by alterations in pulmonary vascular pressures, in bronchial tone and in speed and size of lung inflations, (Widdicombe, 1961). However since the Hering-Breuer inflation reflex in man is relatively weak, being elicited only by inflations considerably larger than normal tidal volumes, others suggest that tonic influence of this reflex in quiet breathing at least in man is unlikely, (Guz, Noble,

Trenchard, Cochrane, and Makey, 1964). In all species however, once a certain threshold volume is reached the inspiratory off-switch provided by slowly adapting stretch receptor input determines tidal volume (V_T) and inspiratory time, (Euler, 1986). As well as exerting some control over inspiratory time in some species including dogs and rabbits, the expiratory discharge of slowly adapting receptors determines expiratory time. Neurophysiological experiments in spontaneously breathing dogs also confirm that expiratory time is determined by the firing frequency of slowly adapting receptors in expiration, (Trenchard, 1977).

Deflation reflex.

Deflation of the lungs causes reflex tachypnea in dogs rabbits guinea pigs and man (Coleridge and Coleridge, 1986). On lung deflation, inspiration and expiration both shorten, and tidal volume is reduced. The effect on V_T is probably due to mechanical restriction but the effects on inspiration are clearly excitatory because peak phrenic activity and minute ventilation increase, (Coleridge and Coleridge, 1986). The reflex is usually blocked at the relatively high temperature of around 0-4°C, (Koller and Ferrer, 1970) and so is thought to be mediated through myelinated afferent fibres. RARs are thought to be responsible for this reflex as they are stimulated by all the different methods used to invoke this reflex, (Coleridge and Coleridge, 1986). Deflation reduces the input from slowly adapting receptors and increases that from rapidly adapting receptors (RARs), (Adrian, 1933). The reflex increase in breathing frequency appears to result from the reduced input from SARs, while the increased inspiratory drive (increased peak phrenic activity) appears to result from stimulation of RARs, (Coleridge and Coleridge, 1986). The primary reflex influence of RARs in the deflation reflex is to increase respiratory drive and lengthen rather than shorten inspiration, (Coleridge and Coleridge, 1986).

Head's paradoxical reflex.

Head's paradoxical reflex observed in rabbits has not been established in man, possibly due to the heroic procedures involved, (Widdicombe, 1961). This reflex was obtained by moderate inflation of the lungs in rabbits whose vagi were rewarming after being packed in ice, (Head, 1889). With cooled vagi, lung inflation evoked a sustained contraction of the diaphragm on which rapid shallow inspiratory movements were gradually superimposed. The effect was abolished when the vagi were cut, (Head, 1889). Since the paradoxical reflex can still be obtained with the vagus nerves cooled to $3^{\circ} - 5^{\circ}\text{C}$, a temperature at which input from SARs and RARs is blocked, it is likely that the reflex is initiated by stimulation of nonmyelinated afferent vagal fibres, although there is probably some RAR activity remaining at such temperatures which may make a significant contribution, (Coleridge and Coleridge, 1986). A reasonable interpretation of Head's paradoxical reflex in rabbits is that pulmonary C-fibres are active during moderate inflation, but when the vagi are at body temperature their excitation effects on inspiration are masked by the more powerful inhibitory input from SARs. So only, when the vagi are cooled are the affects of the C-fibres revealed, (Coleridge and Coleridge, 1986).

Gasp reflex.

A large increase in pump stroke or in airflow delivered to the lungs of ventilated dogs often evokes a reflex increase and prolongation of phrenic discharge and a greatly increased tidal volume, (Coleridge and Coleridge, 1986). These augmented breaths or gasps were identical to the excitatory effects on phrenic activity evoked in cats by large rapid inflations, described by Knowlton and Larrabee, (Larrabee and Knowlton, 1946). This reflex was attributed by these workers to stimulation of rapidly adapting receptors, (Knowlton and Larrabee, 1946). This role for rapidly adapting receptors in initiating the gasp reflex is supported by other workers as the excitatory effects of the gasp reflex disappear at vagal temperatures below 10°C , when conduction in the thinner rapidly adapting fibres is blocked (Widdicombe, 1954). It is unlikely that pulmonary stretch endings are the agent for such gasps as identical augmented breaths, similar in all respects, can be triggered by either pulses of inflation or deflation in inspiration, (Davies and Roumy, 1982), while recordings from stretch fibres have shown that they are only excited by pulses of inflation, (Davies and Roumy, 1982). Also stimulation of stretch receptors in inspiration would decrease rather than increase inspiratory duration. When activity of SARs is blocked by sulphur dioxide this did not suppress triggered augmented breaths, although they were less frequently produced, (Davies and Roumy, 1982). These experimentally induced gasps

may be examples of the same phenomenon as the augmented breaths or sighs that occur spontaneously, and whose incidence increases in various conditions including hypoxia, (Coleridge and Coleridge, 1986).

Static inflations.

Large static inflations of the lung cause breathing movements to become more rapid and shallow, (Coleridge and Coleridge, 1986). It is thought likely that pulmonary C-fibres and to a lesser extent bronchial C-fibres, are the afferent pathway for this reflex, (Coleridge and Coleridge, 1986).

Chemical reflexes.

The pulmonary chemoreflex is a complex pattern of responses that includes bradycardia, hypotension, and apnoea followed by rapid shallow breathing, (Coleridge and Coleridge, 1986). This reflex is evoked by intravenous injections of various foreign chemicals and is produced when the chemicals reach the lungs and stimulate afferent vagal endings, (Coleridge and Coleridge, 1986). Pulmonary C-fibres (J-receptors) are the afferents responsible for initiating the chemoreflex, (Coleridge and Coleridge, 1986).

Airway defence reflexes.

The airway defence reflexes essentially provide protection for the lungs, (Coleridge and Coleridge, 1986). When irritants are inhaled they trigger responses that include coughing and sneezing. These effects are increased and appear to be modified in some respects when the irritants reach the lower airways, (Coleridge and Coleridge, 1986). It has been widely accepted that RARs provide the major afferent input for the airway defence reflexes, (Coleridge and Coleridge, 1986). Many investigations have attributed the rapid shallow breathing and reflex bronchoconstriction evoked by administration of histamine and antigen to stimulation of these receptors, (Coleridge and Coleridge, 1986).

Christie, suggested that the Hering-Breuer inflation reflex may be of importance in the control of breathing in man, and that this reflex may be responsible for adjusting the rate and depth of breathing to make it most mechanically economical, (Christie, 1953). It has also been postulated that the inflation reflex might be of significance in both normal conditions and when the mechanical properties of the lungs are changed, as in lung disease, (Widdicombe, 1961). Widdicombe's investigation however showed the reflex to be relatively weak in man compared to other animals and therefore he suggests caution in ascribing any important role to the Hering-Breuer reflexes

modifying the pattern of breathing in healthy man, (Widdicombe, 1961). A vigorous deflation reflex has also been observed in conscious human subjects although the response to deflation was weak or absent in anaesthetised patients, (Widdicombe, 1961).

Since both the inflation and deflation reflexes have been demonstrated in man they may have a role in controlling breathing in humans, we therefore recorded in this study activity in the lung receptors thought to be responsible for their initiation. The work of this thesis involved recording the activity of the receptors thought to be responsible for the Hering-Breuer inflation and deflation reflexes, the SARs and RARs respectively. SARs and RARs were investigated during eupnoeic breathing and during inflation and deflation procedures. However Head's paradoxical reflex has never been clearly demonstrated in man and since it is supposed that unmyelinated fibres initiate this reflex we did not attempt to record from these fibres. The number of unmyelinated fibres we would have been able to obtain would probably have been small because of the technical difficulty of the procedure, and electron microscopy studies suggest that there is a paucity of unmyelinated fibres in the species we intended to use, that is the rat, (Sant' Ambrogio, 1987). There is also little evidence to suggest that Head's paradoxical reflex might be important in control of breathing patterns in normal or emphysematous animals.

Since my thesis is that alteration of the architecture of the lung alters the activity of pulmonary mechanoreceptors it is relevant to review the nature and normal environment of these mechanoreceptors. The lung surface in humans is very large in the region of 100m^2 . The vast majority of this area is the gas exchange system of the lung alveoli, with only a few square metres being occupied by the conducting airways. Despite this vast respiratory area the afferent nervous supply to the respiratory region is not obvious or conspicuous. Only a few thousand afferent fibres supply the respiratory region. This seems a meagre nervous supply compared with the sensory fibres supplying the 2m^2 of skin surface which are innervated by fibres in excess of 1 million, (Sant' Ambrogio, 1982).

The vagal afferent innervation of the tracheobronchial tree has been demonstrated to be of significant importance, (Sant' Ambrogio, 1982). This has been shown by selective blockade of vagal afferents, which results in marked alterations in the pattern of breathing and loss of the capability to respond to noxious stimuli by coughing, (Sant' Ambrogio, 1982). The activity in several or all types of vagal afferent endings is blocked by anaesthetising airways, (Sant' Ambrogio, 1982); while slowly adapting stretch receptor endings can be selectively blocked using sulphur dioxide (Davies, Dixon, Penman, Widdicombe and Wise, 1978). Both procedures produce

similar although not identical changes in rate and depth of breathing as those observed after cervical vagotomy, (Sant' Ambrogio, 1982). In the SAR blocked state inspiratory duration is less than in the vagotomised state. This is thought to be likely due to RARs having an indirect effect on duration of inspiration, since RAR activity (and unmyelinated fibre activity provoked by rapid inflation or deflation of the lungs) has never been found to directly shorten inspiration, (Davies and Roumy, 1986). After vagotomy the efficiency of respiration is reduced, as measured by the increased work of breathing, (Lim, Luft and Grodins, 1958) and the depressed ventilatory response to carbon dioxide, (Guz, Noble, Widdicombe, Trenchard, Mushin and Makey, 1966). Vagal afferents arising in the tracheobronchial tree are also involved in maintaining patency of the larynx during hypoxia, (Sant' Ambrogio, 1982). Also when conduction in the vagus is blocked after a pneumothorax, blood gases and respiratory mechanics deviate more from the normal values than when the vagal activity is present after a pneumothorax, (Sant' Ambrogio, 1982).

AFFERENT NERVE SUPPLY TO THE TRACHEOBRONCHIAL TREE.

The afferent fibres of the tracheobronchial tree are part of the peripheral autonomic nervous system. There are two components to the afferent innervation of the respiratory system: the parasympathetic and the sympathetic. The parasympathetic (vagal) component is generally considered to have a functionally more important role than the sympathetic counterpart travelling to the spinal cord, (Sant' Ambrogio, 1982).

Vagal afferent endings consist of slowly and rapidly adapting mechanoreceptors supplied by myelinated fibres and the simpler endings of nonmyelinated fibres. The endings of the nonmyelinated C-fibres appear to be of more than one sensory modality, (Coleridge and Coleridge, 1986).

Data from morphological fibre counting studies have shown there to be many more nonmyelinated fibres present than myelinated ones. In cats nonmedullated C-fibres are 3.17 times more numerous than the medullated A-fibres, (Sant' Ambrogio, 1982). In a more recent study on the cat vagus nerve, using light and electron microscopy, the unmyelinated component was found to be 10.8 times more numerous than the myelinated counterpart, (Sant' Ambrogio, 1987). However the relative proportions of these two types of fibres may differ between species, as in the rabbit the nonmedullated C-fibres are decisively in the minority, (Sant' Ambrogio, 1982). Electron microscopy of the alveolar wall in human and rat lungs revealed only a scant number of nonmyelinated fibres and few or no identifiable afferent terminals. In

contrast, the mouse has a good supply of nonmyelinated fibres and recognisable afferent terminals within its alveolar walls and alveolar ducts that are often associated with Type 1 pneumocytes, (Sant' Ambrogio, 1987). This morphological difference highlights the importance of always being aware of the possibility of major species difference and the limits of extrapolating data obtained in one species to another.

Both physiological and morphological data indicate the presence of afferent endings at the level of the gaseous exchange area in the alveoli, (Paintal, 1980), (Meyrick, 1971). The evidence suggests that only the nonmedullated fibres innervate the alveoli walls while both sets of fibres, medullated and nonmedullated are distributed to the airways, (Sant' Ambrogio, 1982). Since endings of the myelinated fibres, SARs and RARs are confined to the airways their distribution is considerably smaller than if they were present in the alveoli: 6-10m² instead of 60-100m² in humans, (Sant' Ambrogio, 1982). SARs can be stimulated by probing when the catheter is large enough to distend the airways, whereas the RARs are also stimulated when the probing instrument touches the luminal surface only lightly; this indicates differences in the location of the receptor endings within the walls of the airways, (Sant' Ambrogio, 1982).

Slowly adapting pulmonary stretch receptors.

In 1933, Adrian postulated that the activity of the slowly adapting pulmonary mechanoreceptors was related to lung volume, (Adrian, 1933). Stretch receptors are responsible for the Hering-Breuer inflation reflex in animals. The Hering-Breuer reflex is not thought to have any influence on the resting pattern of breathing in man as discussed in the section on reflexes. However the activity of the receptors responsible for this reflex are important in determining tidal volume and inspiratory duration, as once a certain threshold lung volume is reached the SARs' input to the medulla switches off inspiration. Not only do SARs determine V_T and T_i through their operation of the inspiratory off switch; but also their activity in expiration, in some species, determine T_e and they thus influence breathing frequency at rest, (Coleridge and Coleridge, 1986). So SARs exert control over both inspiration and expiration, their increasing discharge in inspiration provides an inspiratory off-switch, while their continuing firing during expiration lengthens the respiratory pause, (Sant' Ambrogio, 1982). Another important role of SARs is to promote bronchodilation, (Coleridge and Coleridge, 1986).

The slowly adapting stretch receptors are the more easily identifiable of the endings as they have a respiratory rhythm due to their regular discharge pattern, (Sant' Ambrogio, 1982). SARs are stimulated during both transient changes in lung volume

and during maintained inflations. These receptors increase their rate of discharge in a regular way in the course of inspiration, (Sant' Ambrogio, 1982). SARs can be blocked in rabbits by exposure to sulphur dioxide in a concentration of 300 parts per million for 3 hours, (Davies, Dixon, Penman, Widdicombe and Wise, 1978).

Morphology.

Conventional staining techniques have revealed nerve endings of myelinated fibres that are associated with airway smooth muscle. Degenerative experiments have shown these to be vagal afferents, (Coleridge and Coleridge, 1986). These endings are thought to be SAR terminals. Detailed examination of these endings with the electron microscope show that just before losing its myelin sheath and splitting into terminal branches the axon enlarges and contains many mitochondria. The branches end as free lanceolate terminals rich in mitochondria; they are bound to connective tissue elements between the lamina propria and the smooth muscle layer and are oriented along the long axis of the bronchus, (Coleridge and Coleridge, 1986).

Distribution.

SARs are distributed throughout the tracheobronchial tree down to the terminal bronchioles, (Sant' Ambrogio, 1987) but they are not thought to innervate as far as the alveoli. However it is possible that SARs might be located still more peripherally, as the exact position of the receptors in the airways may be beyond the resolution of the exploring probe used to locate the ending, since lung parenchyma surrounds the bronchial wall so closely that respiratory bronchioles and alveolar ducts may be only a few hundred microns away, (Coleridge and Coleridge, 1986). Most localisation studies indicate a higher concentration of SARs in the larger, more proximal airways with a progressive decline toward the periphery, (Sant' Ambrogio, 1987). The intrapulmonary SARs are believed to play a more important role in the inflation reflex, whereas other reflex responses are attributed to SARs of the extrapulmonary tracheobronchial tree, (Sant' Ambrogio, 1987).

Most of the slowly adapting stretch receptors (SARs) are located in intrathoracic airways, although quite large proportions are found in the extrathoracic trachea, (Sant' Ambrogio, 1987). Activity from SARs has been recorded from vagal filaments and from neurones in the nodose ganglion in several mammals including humans. The intrathoracic SARs increase their rate of discharge during inspiration while those receptors found in the extrathoracic trachea increase their activity during the period of expiratory flow, (Coleridge and Coleridge, 1986). A significant number of the receptors maintain a discharge at functional residual capacity (FRC), (Sant'

Ambrogio, 1987). Those SARs active at the end of expiration are referred to as tonic or low-threshold receptors, whereas those active only during inspiration are called phasic or high-threshold receptors, (Coleridge and Coleridge, 1986). This terminology has been criticised for incorrect portrayal of the activity of the receptors, as "tonic" suggests a discharge at a constant rate rather than a modulated discharge displayed by SARs, (Sant' Ambrogio, 1982). Also the use of "low or high threshold" implies an intrinsic difference in the receptors' level of activation, when their different behaviour may merely reflect a different degree of stimulation related to mechanical factors, (Sant' Ambrogio, 1982). Most of the receptors active at FRC are located in the larger extrapulmonary airways where mechanical factors suggest that the same transmural pressures result in a greater stimulus to the SARs due to a larger circumferential tension as predicted by the Laplace relationship, (Sant' Ambrogio, 1982). Another argument against there being distinct types of stretch receptors is that the fibres have similar conduction velocities, (Sant' Ambrogio, 1982).

A particular class of slowly adapting receptors display an expiratory discharge during spontaneous ventilation, (Luck, 1970). These receptors have myelinated fibres and are known to be localised in the intrapulmonary and extrapulmonary intrathoracic airways, (Luck, 1970) but the stimulus for activation of these receptors is obscure, (Sant, Ambrogio, 1987).

SARs have a sustained discharge in response to a maintained lung inflation. There is an immediate rapid decline in activity after the inflation that slows progressively to a constant firing, (Sant, Ambrogio, 1982). These slowly adapting processes have been found to be related to the viscoelastic properties of the tissue containing the SARs, (Davenport, Sant, Ambrogio and Sant' Ambrogio, 1981).

Patterns of discharge.

The pattern of input from slowly adapting stretch receptors influences the central respiratory mechanism in a phase related manner, (Trenchard, 1977). Therefore the different pattern of receptor discharge from receptors located in different regions of the airways may have functional significance, (Coleridge and Coleridge, 1986). SARs in the trachea firing phasically with each breath will affect the timing of inspiration as they reach peak discharge during inspiration; while their extrathoracic counterparts reaching peak frequency during expiration, when transmural pressure is positive at this site, will have their main effect on expiratory time, (Coleridge and Coleridge, 1986).

Conduction velocities for fibre from both SARs and RARs are consistent with their modulated nature, (Sant' Ambrogio, 1982). The mean conduction velocity of

SARs is somewhat higher than in RARs although there is considerable overlap, (Sant' Ambrogio, 1982).

Physiological stimulus.

Although there is a relationship between respiratory volume and SAR discharge rate the response of these receptors is clearly more closely related with transpulmonary pressure and even more directly to circumferential tension, (Sant' Ambrogio, 1987).

Response to carbon dioxide.

Carbon dioxide can either stimulate or inhibit airway stretch receptors depending on the state of bronchomotor tone, (Sant' Ambrogio, 1987). An inhibitory effect of CO₂ has been shown in several animals including rats, (Sant' Ambrogio, 1987).

Rapidly adapting receptors.

RARs were first recorded from in 1929, (Keller and Loeser, 1929), although the first detailed description of RARs was made by Knowlton and Larrabee in 1946. They observed that large rapid inflations of the lungs produced brief gasps, instead of reflex inhibition of inspiration. They tried to determine whether these opposite effects on inspiratory activity could be explained by the existence of two types of vagal mechanoreceptors with different thresholds to inflation. Receptors with myelinated fibres were identified that were stimulated by inflation but were significantly different from SARs, (Knowlton and Larrabee, 1946). Rapidly adapting receptors are less numerous than SARs. In cats the SARs outnumber the RARs by a factor of 10, (Widdicombe, 1954 b). In the rabbit SARs are also found more frequently, 4 times more often than RARs, (Sant' Ambrogio, 1982).

Investigators have been willing to accept that activity from RARs may contribute to patterns of breathing in pathological states, (Mills, Sellick and Widdicombe, 1969) and that they are partially responsible for gasps and sighs, (Knowlton and Larrabee, 1946). However most reports do not seem to embrace idea of a role for RARs in the normal control of breathing or indeed that they have any significant activity in eupnoea, (Bergren and Sampson, 1982). However in rabbits and dogs if V_T or airflow increases activity of these receptors increases markedly, (Coleridge and Coleridge, 1986). When tidal volume is increased in ventilated rabbits, the activity of RARs is suggested as the likely vagal inspiratory initiation influence when SARs are blocked with SO₂, (Davies, Sant' Ambrogio and Sant' Ambrogio,

1981). Brief intense bursts of irritant receptor activity provoke an augmented inspiration, (Davies and Roumy, 1978), although they have been indicated to also play a role in shortening expiration, (Davies, 1978).

The receptors described by Knowlton and Larrabee have a higher volume threshold than SARs, a more rapid rate of adaptation and a more irregular pattern of discharge, (Coleridge and Coleridge, 1986). The receptors were named rapidly adapting receptors due to their characteristic rapid adaptation to a stimulus. They are also known as irritant receptors as they have a notable response to irritants such as ammonia, dust and cigarette smoke in the lungs, (Mills, Sellick and Widdicombe, 1969). Receptors at the carina are sensitive to irritants with a particulate nature and are therefore believed to be cough receptors, (Sant' Ambrogio, 1982). Those in the intrapulmonary airways are thought not to cause cough. RARs adapt very rapidly to maintained inflation, (Coleridge and Coleridge, 1986). RARs are also stimulated by forced deflation where they respond with an irregular maintained increase in firing, (Coleridge and Coleridge, 1986).

Morphology.

RARs are thought to correspond to the epithelial nerve endings identified in the respiratory airways, (Larsell, 1921). The epithelial endings are the terminal arborizations of myelinated fibres that ramify in the tracheobronchial submucosa, (Coleridge and Coleridge, 1986). They appear to be more prevalent at points of bronchial branching, (Sant' Ambrogio, 1982). The main axons of the receptors in the trachea and bronchi branch extensively to supply a wide area of mucosa, (Larsell and Dow, 1933).

Distribution.

Like SARs, RARs are only found in areas of lung parenchyma with bronchi and bronchioles. They are not evenly distributed along the tracheobronchial tree but are more concentrated in the more proximal airways, (Martolla, Sant' Ambrogio and Clement, 1975). In the extrapulmonary airways the concentration of RARs increases from the upper trachea to the main stem bronchus, (Sant' Ambrogio, 1987).

Patterns of discharge.

RARs adapt very rapidly to maintained inflation, producing an irregular and brief burst of activity which decreases within one second to 20% or less of the initial discharge, (Coleridge and Coleridge, 1986). The discharge pattern, although generally related to the respiratory cycle, is markedly irregular as the number of action potentials occurring in each cycle are reported to vary considerably, (Armstrong and Luck, 1974), (Sampson and Vidruk, 1975), (Sellick and Widdicombe, 1969).

Physiological stimulus.

Most accounts give the general impression that rapidly adapting receptors are virtually inactive in eupnoea, (Coleridge and Coleridge, 1986). However RARs may give useful signals of airflow rates, because with successive lung inflations at increasing rates of flow within the physiological range the firing threshold decreases and the impulse frequency at each volume increment increases, (Pack, 1981). RARs may also have a role in influencing the onset of inspiration along with the waning central inhibition from SARs, (Davies, Sant' Ambrogio and Sant' Ambrogio, 1981). The RARs may actually provide an inspiratory initiation drive, (Davies, Sant' Ambrogio and Sant' Ambrogio, 1981). This role is compatible with the effects of irritant receptors in shortening expiration and provoking augmented breaths if the initiation of inspiration is considered as a termination of expiration.

Response to carbon dioxide.

RARs in dogs increase their activity when airway CO₂ is reduced, (Coleridge, Coleridge and Banzett, 1978). When CO₂ was raised to normocapnic levels excitation of RARs occurred, although increase in CO₂ concentration above normocapnic levels did not modify RAR activity.

Fibres from rapidly adapting receptors have conduction velocities similar to those from SARs; although the mean velocity is somewhat lower in the RARs than in the SARs, (Sant' Ambrogio, 1987). These conduction velocities of the pulmonary receptors have been measured in several different species, (Sant' Ambrogio, 1987).

C-Fibre receptors.

C-fibre afferents do not correspond to a homogenous group of receptors, (Sant' Ambrogio, 1987). They have been separated by physiological and pharmacological criteria into two main categories: pulmonary C-fibre receptors also known as juxtapulmonary capillary receptors, (J receptors) and bronchial C-fibre receptors, (Sant' Ambrogio, 1987). This nomenclature relates to the respective anatomical sites

of the fibre endings they are names which merely acknowledges the blood supply of the endings. Stimulation of pulmonary C-Fibres causes rapid, shallow breathing that is often preceded by a brief interval of apnoea, (Sant' Ambrogio, 1987).

Morphology.

Vagal degeneration experiments in cats have established that nonmyelinated afferents greatly outnumber myelinated, (Sant' Ambrogio, 1982). Unmyelinated fibres with corresponding terminals have been found within the tracheal epithelium of humans. In mice similar findings have been made in the intrapulmonary airways where the endings have structures identical to those of the alveolar wall (Sant' Ambrogio, 1987). In rats electron microscopy studies reveal only a scant number of nonmyelinated fibres in the alveolar wall, (Sant' Ambrogio, 1987).

Distribution.

J receptors appear to be located near the pulmonary capillaries based on evidence that they are accessible to chemicals, (Coleridge and Coleridge, 1986).

Patterns of discharge.

During spontaneous breathing, pulmonary C-fibre endings show significant activity with a respiratory rhythm of (1.9 ± 0.2) impulses per second). This activity decreases markedly under conditions of artificial ventilation with the chest open. The activity of bronchial C-fibre receptors is sparse and irregular during spontaneous and artificial breathing, (Sant' Ambrogio, 1987).

Physiological stimulus.

During eupnoeic breathing pulmonary and bronchial C-fibres discharge irregularly and at low frequencies, (Coleridge and Coleridge, 1986). Pulmonary C-fibres are stimulated in dogs and cats by lung inflation, (Coleridge and Coleridge, 1986). Bronchial C-fibres in dogs are less sensitive to lung inflation and indeed many are unaffected by even extreme hyperinflation. Deflation is only at best a weak stimulus to both C-fibres from both the lung and airways, (Coleridge and Coleridge, 1986).

Pulmonary C-fibres in cats and dogs are stimulated by pulmonary congestion, (Coleridge and Coleridge, 1986). C-fibre discharge increases linearly with left arterial pressure when congestion is induced by progressive inflation of a balloon in the left atrium. Paintal suggests that such a response may occur in exercise and that the stimulus provided by exercise gives these afferent nerve endings their most significant physiological role, (Paintal, 1969). This is supported further by the observation that

the discharge of these receptors increases when pulmonary blood flow to the appropriate lobe is increased by obstructing flow to the other lobes, (Anand and Paintal, 1980). Bronchial C-fibres are generally much less sensitive to pulmonary congestion. Since Pulmonary C-fibres (J receptors) respond to congestion and oedema they may be involved in limiting the intensity of exercise, (the J reflex), (Paintal 1969).

Response to carbon dioxide.

It has been suggested that pulmonary nerve endings supply a signal proportional to the CO_2 content of mixed venous blood, (Coleridge and Coleridge, 1986). A reflex increase in ventilation occurs when CO_2 is added to the inspired air, (Coleridge, Coleridge and Banzett 1978). In spontaneously breathing dogs; in which pulmonary and systemic circulations are perfused separately and CO_2 is delivered to the lung by the normal route, (pulmonary arterial blood), ventilation increased by approximately 75% when pulmonary CO_2 was increased from 35-80 mmHg, (Coleridge and Coleridge, 1986). The response was found to be abolished by vagotomy, (Coleridge and Coleridge, 1986). Since SARs are inhibited by very low PCO_2 , changes in the activity of this group of afferents seem unlikely to account for the reports of vagal reflex hyperpnea when pulmonary PCO_2 increases above 40 mmHg. Hence it is thought that the response is likely to be due to the activation of an excitatory input, rather than to be due to a disinhibition that results from a CO_2 -dependent reduction of SAR discharge. From results of vagal cooling where CO_2 induced tachypnea is maximal at temperatures of 8-4°C when SARs are blocked it has been suggested that afferent C-fibres mediate this change in breathing frequency evoked by breathing CO_2 , (Phillipson, Fishman, Hickey and Nadel, 1973).

VAGAL AFFERENT CONTROL OF BREATHING

The influence of vagal lung receptors to the regulation of breathing have been studied. The duration of inspiration appears to be governed by two distinct mechanisms: (Clark and von Euler, 1972)

- 1). The bulbo-pontine pace-maker mechanism which is active in the absence of volume-feedback or when the threshold level of volume-feedback is high. This mechanism is therefore controlling inspiratory duration in man at low volumes, and in vagotomised animals.

2). When volume-feedback is functioning the inspiratory duration is dependent on lung volume. The relative differences between conscious man and anaesthetised cats seem to be quantitative rather than qualitative, (Clark and von Euler, 1972).

Stretch receptor activity is important in breathing regulation as a measure of lung volume and is a central part of the von Euler model of control of breathing, which is perhaps the most generally accepted of models today. In this, afferent pulmonary stretch receptor activity operates an off switch, switching off inspiration when a certain level is reached. The threshold at which this switch operates falls during each respiratory cycle, so a smaller lung volume is required late in the cycle than early to terminate inspiration. The duration of expiration was postulated to depend on the level of pulmonary stretch receptor activity at the end of inspiration. Therefore the duration of inspiration is linked to the subsequent expiration.

The most commonly agreed theory of control of pattern of breathing by vagal afferents has developed from the work of Clark and von Euler (1972). In this theory slowly adapting pulmonary stretch receptor activity plays a major role, and the role of these receptors in disease can therefore be expected to be significant. Stretch receptors are activated by lung inflation, cut short inspiration but have little effect on the steepness of the inspiratory ramp, and stimulate expiratory activity. They are thought to be excited by the amount of lung expansion, although increasing the rate of lung filling also has some stimulatory action.

The altered pattern of breathing on vagotomy is usually attributed to the removal of tonic activity by slowly adapting receptors, (Widdicombe, 1961). However this cannot be the whole story since blocking stretch receptors with sulphur dioxide would cause animals to display the same breathing pattern as vagotomised animals, which they do not. With stretch receptor block, inspiratory time increases and expiratory time decreases; but when animals are vagotomised a further lengthening of inspiratory time occurs, and a lengthening rather than any further shortening of expiration occurs compared to the stretch receptor blocked state. This is attributed mainly to the input remaining from RARs shortening T_e in the stretch receptor blocked state, which is removed by vagotomy thus producing a lengthening of T_e in the vagotomised state, (Davies, Dixon, Callanan, Huszczuk, Widdicombe and Wise, 1978). From vagal cooling experiments in dogs, C-fibres are also thought to be important in limiting T_e , (Pisarri, Yu, Coleridge and Coleridge, 1986). Therefore T_e lengthening following vagotomy might also be due to the removal of the T_e shortening influence from the C-fibre component of the vagus. In sulphur dioxide block one of the major controls of breathing has been unplugged. The pattern left is the result of rapidly adapting receptors and C- fibres modifying the basic, brain stem

generated pattern displayed when the vagi are cut. With the vagi cut all that remains is the brain stem pattern of breathing being modified by chemoreceptor input and higher centres.

CHEMICAL CONTROL OF BREATHING.

Changes in breathing pattern, produced by changes in lung architecture brought about by emphysema, cannot be considered independently of changes in chemical composition of the blood as both the two are interrelated. Indeed the pattern of breathing influences the chemical composition of blood and *visa versa*. Changes in pattern of breathing due to emphysema can lead to changes in the chemical composition of the blood which then alters the chemical control of breathing.

CENTRAL CHEMORECEPTORS.

Chemoreceptors in the brain show ventilatory response to changes in pH and CO_2 , (O'Regan and Majcherczyk, 1982). When all peripheral chemoreceptors are denervated, animals continue to show increased ventilation when made to breathe carbon dioxide, (O'Regan and Majcherczyk, 1982). Such investigations indicate that receptors or areas within the brain are excited by acidity or changes in carbon dioxide, (Bruce and Cherniack, 1987). No central chemoreceptors have been identified unambiguously but there is ample evidence that chemical, electrical and thermal stimuli applied locally to the ventrolateral shell (VMS) of the medulla will affect respiration, (Bruce and Cherniack, 1987). There may also be other central chemoreceptor cells elsewhere in the brain as for example cells in the DRG have been shown to respond to CO_2 , (Bruce and Cherniack, 1987). The central chemoreceptors, like the peripheral chemoreceptors, have inputs into the respiratory centre of the brain, (O'Regan and Majcherczyk, 1982).

The respiratory neurones in the medulla receive information on the level of arterial PO_2 and PCO_2 in the body from the peripheral chemoreceptors (carotid and aortic bodies), (Cherniack, 1991).

PERIPHERAL CHEMORECEPTORS.

The carotid and aortic bodies make up the peripheral chemoreceptor system, (O'Regan and Majcherczyk, 1982). The carotid bodies lie near the carotid sinuses and several aortic bodies lie adjacent to the aorta. Most investigations have been carried out on the carotid bodies due to their ease of isolation. They are all highly vascularised bits of tissue, loaded with a variety of amines and neuropeptides. The peripheral chemoreceptors consist of groups of cells (type I and type II) surrounding a blood vessel and supplied by an afferent nerve, (Eyzaguirre, C., Zapata, P. 1984). The type I cell is thought to be the actual chemosensitive part of the organ, while the type II cells serve as a support unit, (Cherniack, 1991).

The carotid body is sensitive to changes in PCO_2 , PO_2 , pH, temperature and metabolic poisons, (O'Regan and Majcherczyk, 1982). It is thought that central chemosensitive structures can modify by neural pathways, the peripheral chemoreceptors and visa versa, (O'Regan and Majcherczyk, 1982).

BEHAVIOURAL CONTROL OF BREATHING.

Behavioural control of breathing is well established, and can be exerted by cortical action over anatomic pathways separate from those used by medullary neurones. Higher brain centres have major effects on breathing through voluntary acts, such as talking, which alter respiratory patterns, and changes in state between wakefulness and sleep, (Cherniack and Altose 1987). Sleep has a depressant action on breathing and responses to CO_2 and hypoxia. Sleep can alter responses to reflexes, (Strohl, Cherniack and Gothe, 1986). For example, laryngeal stimulation, which can give rise to coughing during wakefulness, can produce apnoea if it occurs during sleep, (Strohl, Cherniack and Gothe, 1986). The pattern of breathing is also altered by sleep. In general, breathing becomes more regular during non-REM sleep but more erratic in REM sleep. Airway resistance tends to increase during sleep (Strohl, Cherniack and Gothe, 1986).

Changes in mental activity can alter breathing even when awake as well as during sleep. Mental arithmetic stimulates breathing while closing the eyes decreases breathing, (Cherniack and Altose, 1987). Tranquillisers, alcohol and opiates all depress breathing, presumably this is largely to do with their effects on higher brain centres, (Santiago and Edelman, 1985).

In the conscious state humans may control their level of breathing not only automatically but also in part behaviourally to minimise respiratory sensation. In healthy subjects increases in ventilation both from hypercapnia and exercise result in

linear increases in intensity of dyspnoea, (Chonan, Mulholland, Leitner *et al.* 1990). However increases in breathing are associated with a reduction in sense of effort by the equation ($u = P^{1.3} \times T_i^{0.56}$), where P is the pressure produced at the airway and T_i is inspiratory time. The equation shows that pressure at the mouth is the major factor determining sense of effort. Because mouth pressure decreases as tidal volume is reduced, at the same level of minute ventilation, the sense of effort may be minimised by breathing rapidly and shallowly, (Killian, Bucens, and Campbell, 1982). It has also been shown that in healthy subjects restraining breathing produces augmentation of dyspnoea at a constant level of PCO_2 , (Chonan, Mulholland, Cherniack and Altose, 1987). The results of such studies indicate the possibility that conscious humans may adjust their breathing pattern to minimise the respiratory sensation of dyspnoea. Breathing patterns might be regulated to minimise the sensation of effort (Killian, Bucens and Campbell, 1982) and the work of breathing and force output, (Mead, 1960). Since restraining breathing augments dyspnoea in healthy subjects, patients with emphysema may also control their breathing pattern behaviourally to limit their sensation of dyspnoea. It could be possible that people with emphysema might consciously increase their breathing frequency as to breathe more slowly may make their feeling of breathlessness more severe. Patients would try to avoid such breathing patterns and situations which induced or augmented their dyspnoea.

CONTROL OF BREATHING IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

The control system functions sufficiently well in most patients with COPD such that normal blood gas tensions of CO_2 are maintained, (Rebuck and Slutsky, 1986). A control model has been proposed in which ventilation is controlled to minimise a total cost consisting of chemical stimulation and the work of breathing where the cost is the sensation of dyspnoea, (Chonan, Cherniack, Altose, 1991).

INVOLVEMENT OF VAGAL AFFERENTS IN THE PATTERN OF BREATHING SEEN IN LUNG DISEASES.

Patients with pulmonary fibrosis usually present with increases in minute ventilation and breathing frequency that are not chemically mediated, (Lourenco, Turino, Davidson and Fishman, 1965). The characteristic decreased lung compliance associated with this disease could be expected to produce an increase in airway vagal receptor activity, (Savoy, Dhingra and Anthonisen, 1981). However the results of a study using inhaled lignocaine airway anaesthesia to block airway reflexes in humans

with fibrotic lung disease suggest that airway receptors do not contribute in a major way to the control of breathing in pulmonary fibrosis, (Savoy, Dhingra and Anthonisen, 1981). These results assume the effectiveness of the vagal block on all vagal receptors as anaesthesia was assumed to be effective on abolition of a threshold cough response, (Savoy, Dhingra and Anthonisen, 1981), and vagotomy could not be performed in this investigation in human subjects.

It is thought that some patients with inherited differences in respiratory apparatus sensitivity, (Collins, Scoggin, Zwillich and Weil, 1977) may have an increased excitatory input from pulmonary receptors affected by the disease process, which could be responsible for their extreme dyspnoea, (Bradley, Hale, Pimble, Rowlandson and Noble, 1982). Vagotomy and local anaesthetic block of the vagus nerve in humans depresses the frequency of breathing and relieves breathlessness in certain patients with lung disease, (Guz, Noble, Eisele and Trenchard, 1969). Right vagotomy below the origin of the recurrent laryngeal nerve, (to avoid paralysis of the vocal cord on that side), sometimes appears to remove an influence preventing slow deep breathing and exacerbation of dyspnoea, (Bradley, Hale, Pimble, Rowlandson and Noble, 1982).

Lung inflation induced stimulation of pulmonary stretch receptors is thought to perhaps provide an inhibitory effect on the sensation of dyspnoea, (Chonan, Cherniack and Altose, 1991) because a reduction in thoracic displacement intensifies dyspnoea, (Chonan, Mulholland, Cherniack and Altose, 1987).

Lung irritant receptors contribute to the reflex hypernoea and bronchoconstriction in the conditions of pulmonary microembolism, anaphylaxis and drug induced bronchoconstriction, (Mills, Sellick and Widdicombe, 1969). Stimulation of irritant receptors or C-fibres appears to augment dyspnoea because it is accentuated by inhalation of histamine or PGCE₂, (Kikuchi, Taguchi, Hida *et al.* 1989).

PATTERN OF BREATHING IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE.

1. ANIMALS.

Studies in horses with emphysema show inspiratory flow rates to be increased, tidal volumes to be decreased, and intrathoracic pressure changes to be greater than in normal horses, (Gillespie, Tyler and Eberly, 1966). Artificially produced pulmonary embolism, pneumonia, granuloma and collapse in animals causes hyperventilation with a particularly marked rise in frequency of breathing. This is abolished by vagotomy or local anaesthetic block of the vagus nerves which prevents information from the lungs reaching the brain, (Guz and Trenchard, 1971), (Trenchard, Gardner and Guz, 1972), (Phillipson, Murphy, Koser, and Schultze, 1975).

In anaesthetised rabbits with elastase induced emphysema the breathing frequency was found to be lower than the control group of animals, (Delpierre, Fornaris and Payan, 1985). This is in contrast to the rapid shallow breathing observed in conscious humans with emphysema. However very little work has been done into studying the breathing pattern of other animals with emphysema and it is not known if Delpierre's reduction in breathing frequency with emphysema in rabbits occurs in other species.

2. HUMANS.

Patients with COPD generally have an increased ventilatory drive, and they also tend to exhibit rapid, shallow breathing relative to the normal breathing pattern, (Rochester, 1991). These altered breathing patterns are most obvious when breathing is measured from body surface movements, using the technique of respiratory inductance plethysmography to avoid the effects of a mouth piece on breathing, (Tobin, Chadha, Jenouri, Birch, Gazeroglu and Sackner, 1983). Both eupcapnic and hypercapnic COPD patients have higher respiratory rates than do healthy subjects, (Loveridge, West, Anthonisen, and Kryger, 1984), (Loveridge, West, Kryger and Anthonisen, 1986), (Tobin, Chadha, Jenouri, Birch, Gazeroglu and Sackner, 1983). The tidal volume of patients with COPD, both normo and hypercapnic, shows an increase in some studies, (Tobin, Chadha, Jenouri, Birch, Gazeroglu and Sackner, 1983) and in another study there was no difference in the tidal volume of the COPD compared to the controls, (Loveridge, West, Anthonisen, and Kryger, 1984). COPD patients with more airflow limitation, hypercapnia or both have even higher breathing

frequencies and smaller tidal volumes, (Javaheri, Blum and Kazemi, 1981), (Loveridge, West, Kryger and Anthonisen, 1986).

COPD patients with hypoxaemia alone have increased respiratory rates with normal tidal volumes, (Parot, Miaara, Milic-Emili and Gautier, 1982). When bronchoconstriction increases or respiratory failure develops the rapid, shallow breathing of COPD patients becomes more pronounced and parallels increases in neural drive to breathe, (Aubier, Murciano, Fournier, *et al.* 1980), (Oliven, Cherniack, Deal and Kelsen, 1985). Rapid, shallow breathing also develops when COPD patients exercise, particularly when they retain carbon dioxide, (Gimenez, Servera, Candina, *et al.* 1984).

Inspiratory muscle performance is effected by the rapid and shallow breathing pattern, (Rochester, 1991). Since the expiratory time is shortened with this pattern of breathing the respiratory system is prevented from falling back to its relaxation volume; this contributes to hyperinflation and mechanical disadvantage, (Rochester, 1991).

SENSATION.

DYSPNOEA.

One of the most common symptoms of chronic obstructive pulmonary disease is that of breathlessness, shortness of breath or dyspnoea, (Chonan, Cherniack and Altose, 1991). Dyspnoea is, literally, disordered breathing (from the Greek dys = abnormal or disordered, and pnoia = breathe), it is considered by some authors to be different from the sensation of physiological breathlessness.

SIGNIFICANCE OF DYSPNOEA AS A SYMPTOM IN EMPHYSEMA.

In normal conditions breathing occurs without conscious effort. When difficulty with breathing is sensed it is known as dyspnoea. Dyspnoea is considered to be a different sensation to that of physiological breathlessness as occurs during exercise or excitement, (Burki, 1980). The term dyspnoea is reserved for the pathological distressing sensation of laboured breathing felt in many cardiopulmonary disease states. Almost all patients with chronic obstructive pulmonary disease have difficulty in breathing, which limits activity and greatly reduces their quality of life. When the extent of pulmonary dysfunction is mild to moderate, or the patient is at rest, the awareness of breathing may not be present until the patient moves or performs exercise, (Chonan, Cherniack and Altose, 1991). As the disease progresses dyspnoea may become symptomatic and is then difficult to eliminate or reduce unless pulmonary

function, respiratory muscle performance, or physical condition is improved, (Altose, 1985).

Some patients with chronic lung disease are very dyspnoeic in association with an apparently normal responsiveness of ventilation to respiratory stimuli. An inherited difference in the sensitivity of their respiratory apparatus in either the brain stem or peripheral chemoreceptors has been evoked to explain this, (Collins, Scoggin, Zwillich and Weil, 1977), (Bradley, Hale, Pimble, Rowlandson and Noble, 1982). The significance of dyspnoea to the present study is that sensation of breathing is probably mediated by the same afferent systems that mediate the reflex control of breathing and which may be perturbed by changes in lung architecture.

ORIGIN OF DYSPNOEA.

Studies of respiratory sensation in relation to dyspnoea.

1. Breath holding.

Breath holding has been described as an extreme form of the dyspnoeic sensation, (Burki, 1980). The similarity of this sensation to that experienced by patients suffering from chronic obstructive pulmonary disease has led to the breath holding sensation being studied extensively.

The respiratory distress produced by breath holding develops with time. It is also directly related to the arterial PCO_2 and inversely related to PO_2 and lung volume, (Burki, 1980). The distress of breath holding can be relieved, to an extent, by a short period of rebreathing, or even by breathing a gas mixture with a lower PO_2 and a higher PCO_2 than at the break-point of breath holding, (Burki, 1980). This is shown by the subjects ability to perform a subsequent breath hold. These findings suggest that although blood gases and therefore chemically mediated drive to breathe, play a part in generating the sensation of dyspnoea which limits breath holding they do not appear to be the only factor involved. The distress experienced with breath holding is abolished by bilateral vagal nerve block, (Guz, Noble, Widdicombe, Trenchard, Mushin and Makey, 1966), and by curare induced muscular paralysis, (Burki, 1980). During the breath hold the distress occurs simultaneously with the onset of contraction of the diaphragm. An interpretation of these findings is that it is the diaphragmatic contractions, resulting from an increased drive to breathe from both vagal and chemical stimuli, that produce the distressing sensation felt when a breath is held, (Burki, 1980). Studies of the effects in healthy subjects of constraining thoracic

displacement voluntarily, during hyperoxic hypercapnia suggest that sensory feedback originating from mechanoreceptors in the lung, chest wall, or both in response to displacement of the thorax decreases dyspnoea by their action on brainstem respiratory neurones, or on higher brain centres or both, (Chonan, Mulholland, Cherniack, Altose, 1987). This explains why dyspnoea was worse at a given level of PCO_2 when the ventilation was reduced below the spontaneously adopted level. Thus dyspnoea felt by restraining breathing, as in breath holding, could be due to a loss of inhibitory feedback, (Chonan, Cherniack and Altose, 1991).

2. External loads.

Perception of added respiratory loads have been used to study dyspnoea, (Burki, 1980). An increase in the degree of breathlessness, at any given level of ventilation during exercise in healthy subjects, was reported when an external resistive load to inspiration was applied, (Altose, 1985). Intensity of this sensation of breathlessness was related by the same workers to the level of respiratory efferent activity. Anaesthesia of the airways, chest wall block by spinal anaesthesia or mechanical restriction, and vagal block have no effect on detection thresholds, (Burki, 1980). It is possible, however that sites other than the diaphragm may be involved in the perception of load when the diaphragm is abnormal or diseased. Also the sensation of resistive loading is not identical to dyspnoea. Expiratory resistive loading as well as inspiratory loading produces dyspnoea in proportion to the expiratory motor output, (Chonan, Altose and Cherniack, 1990). From the results of such studies the size of the motor command to the respiratory muscles has been postulated to be a major factor responsible for dyspnoea.

3. Awareness of ventilation.

Large increases in ventilation frequency due to chemical stimulation may go unnoticed in healthy subjects, (Burki, 1980). It has also been shown that patients with COPD and breathlessness do not have a significantly different minute ventilation from non-breathless patients, (Burki, 1980). This indicates that awareness of increased breathing frequency cannot be the main factor producing dyspnoea.

4. Chest wall block.

Afferent and efferent information from, and to the intercostal muscles is not thought to cause the sensation of breathlessness, as when these information pathways are blocked no change in the detection threshold for added resistance, or on breath holding time, or sensation and CO₂ response to rebreathing occurs, (Burki, 1980).

5. Vagal afferent studies.

Right vagotomy was found to give symptomatic relief of dyspnoea to four out of five patients with emphysema. Exercise ventilation was not noticeably depressed but pattern of breathing altered, (Bradley, Hale, Pimble, Rowlandson and Noble, 1982). Breathing after vagotomy became deeper and the rise in frequency with exercise was depressed. Vagotomy appeared to remove an influence preventing slow, deep breathing and exacerbation of dyspnoea during exercise, (Bradley, Hale, Pimble, Rowlandson and Noble, 1982). It is possible that some patients have an increased excitatory input from pulmonary receptors affected by the disease process, (Bradley, Hale, Pimble, Rowlandson and Noble, 1982).

Guz and co-workers reported that bilateral blockade of the vagus and glossopharyngeal nerves, produced by locally administered lignocaine, prolonged maximum breath-holding time with alleviation of the associated distress, although the detection of added loads to the airways was unaffected, (Guz, Noble, Widdicombe, Trenchard, Mushin and Makey, 1966).

Kelsen and colleagues found the sense of effort to breathe was greater in response to bronchoconstriction than with external loading which produced the same level of resistance to breathe, (Kelsen, Prestel, Cherniack, *et al.* 1981). This suggests that different mechanisms were involved in the production of the sensation. Alternatively the differences noted could be due to mechanoreceptors, probably in the airways, supplied by the vagus nerves, responding differently depending on the site of

increased resistance. Support for a contribution to the sensation of dyspnoea by vagal afferents comes from work by Taguchi and colleagues where airway anaesthesia decreased dyspnoea induced by histamine at a given level of airway resistance but did not effect dyspnoeic sensation during external loading, (Taguchi, Kikuchi, Hida, *et al.* 1989). This work also supports the idea that different components of the sensation of dyspnoea exist, or that different forms of dyspnoea occur.

Rapidly adapting and C-fibres are thought to contribute to the sensation of breathlessness at least in health. When lung receptors are stimulated, in healthy humans by prostaglandin E₂, airway resistance and lung volume are unchanged at a given level of exercise yet the subjects dyspnoea increases, (Kikuchi, Taguchi, Hida *et al.* 1989).

Complete vagal block by lignocaine, when intercostal muscle and diaphragmatic innervation remain intact allowed a prolongation of the breath holding time and a higher PCO₂ at the breaking point of the manoeuvre, (Guz, Noble, Widdicombe, Trenchard, Mushin and Makey, 1966).

An extreme exertional dyspnoea associated with a rapid, shallow breathing pattern, in a patient with unilateral pulmonary venous obstruction, was markedly reduced with the removal of afferent vagal activity that resulted from sectioning of the right thoracic vagus. Breathing pattern and functional status of the patient was also improved following right vagotomy and the patient's exertional dyspnoea resolved completely, (Davies, McQuaid, Iber, *et al.* 1987). Davies and co-workers concluded from these results that vagal afferents were the cause of the severe dyspnoea of this patient.

Since in healthy subjects a reduction in thoracic displacement intensifies the sensation of dyspnoea, (Chonan, Mulholland, Cherniack and Altose, 1987), and thoracic displacement, which normally occurs in inflation, stimulates stretch receptors, it has been suggested that stretch receptor activity may have an inhibitory effect on dyspnoea. This presumes that in the converse situation to that of Chonan and colleagues, where there is increased stimulation of stretch receptors by more thoracic displacement a reduction in the sensation of dyspnoea would result. Another factor to consider is that in these experiments joint receptors in the ribs and chest wall and muscle spindles in the intercostal muscles would be providing a modified input to the brain, due to restraining chest movements which could have provided the distressing sensation. A modified pattern of total activity coming up the vagus would be present in these studies, as not only would the stretch receptor component be altered but also a large modifying component could be due to rapidly adapting receptors, as they

would be stimulated by actelecticis brought about by restraining spontaneously adopted breathing movements.

Among the various airway receptors, each type may play a different role in the sensation of dyspnoea. Stimulation of pulmonary stretch receptors by lung inflation may have an inhibitory effect on the sensation of dyspnoea because, as mentioned above, a reduction in thoracic displacement intensifies dyspnoea. In comparison, stimulation of rapidly adapting receptors, or C-fibres, or both appears to augment dyspnoea as it is accentuated by inhalation of histamine or PGE₂ (Kikuchi, Taguchi, Hida *et al.* 1989).

Sensation of chest tightness was shown to be possible when the only nervous communication between the thorax and the central nervous system was via the vagus nerve, all other such pathways being destroyed by section of the third cervical segment of the spinal cord, (Howell, 1969). Patterson and co-workers' 1962 study supports a role of vagal afferent information in dyspnoea sensation since it was induced in a patient with complete paralysis up to the level of C1- C2 when CO₂ was inhaled, (Patterson, Mullinax, Bain, *et al.* 1962). However other studies on similar neurological disease states could find no evidence of CO₂ induced dyspnoea and in fact reported increased breath holding time when spinal cord transection was at the level of C3, (Burki, 1980). Therefore afferent and efferent nervous information from the chest wall and or diaphragm may contribute to dyspnoea, since the breath holding could be prolonged when their contributory information was not being transmitted. These results are not necessarily contradictory rather they show that dyspnoea is not a single sensation and suggest that one of its components can arise from vagal receptors in the bronchial tree. Another component is presumably caused by change in pulmonary mechanics and can be detected by somatic mechanisms.

Although the sensation of dyspnoea itself cannot be studied in animal models, the mechanism of the likely vagal component of this sensation could be investigated. Studying the activity of lung mechanoreceptors in an animal model of the disease could help give an insight into receptor activity changes in human emphysema, possibly accounting for changes in pattern of breathing and sensation in this condition.

Possible origin of dyspnoea.

Dyspnoea is accentuated by higher levels of CO₂ even when levels of ventilation are similar, (Chonan, Mulholland, Leitner, *et al.* 1990). There may be different forms of dyspnoea and there may be several mechanisms that bring about this very distressing breathing sensation for each of the possible forms. There might be several mechanisms contributing towards the one sensation. Each mechanism may have a

larger or smaller contribution to the total sensation, depending on the fundamental reason for the distress. Patients with hypoxaemic dyspnoea due to advanced cancer had a much more consistent benefit from oxygen than similarly hypoxaemic patients with dyspnoea due to pulmonary disease, (Bruera, de Stoutz, Velasco-Leiva, Schoeller, Hanson, 1993). This could indicate a different mechanism for the dyspnoea in the two diseases. Altose observed there to be no difference between the intensity of dyspnoea felt by COPD and healthy subjects when the respiratory output was the same, by scaling the dyspnoea felt by the two groups at a given occlusion pressure corrected for maximum inspiratory muscle strength, (Altose, 1985). The physiological breathlessness experienced on exertion, and the breathlessness felt by normal subjects during breath holding and increased load breathing situations may have different underlying mechanisms to the pathological breathlessness felt by severely ill COPD patients.

The two classical groups of chronic obstructive pulmonary disease patients, the "pink puffers" and the "blue bloaters" suffer dyspnoea to different extents. The major symptom of the pink puffers is extreme dyspnoea, (Crofton, and Douglas, 1981) although their arterial blood gas tensions reveal a PO_2 close to normal and a PCO_2 which is typically reduced. The arterial hypoxaemia is insufficient to cause cyanosis and the patients therefore appear pink. This is achieved by a high level of total ventilation, hence the patient is pink and puffing. However dyspnoea does not appear to be the major symptom of the blue bloaters despite PO_2 being reduced and the PCO_2 raised due to mismatch of ventilation and perfusion, (Crofton and Douglas, 1981).

Muscle receptors in respiratory muscles have been associated to the occurrence of dyspnoea, (Chonan, Cherniack and Altose, 1991). Two types of mechanoreceptors in respiratory muscles that influence respiratory control are muscle spindle endings which are considered to be length receptors and tendon organs, which are force receptors. These may act on medullary respiratory neurones or through direct projection to the cerebral cortex, (Shannon, 1986). Muscle spindles have been shown to have inhibitory effect on inspiratory neurones.

The pathological breathlessness of dyspnoea is experienced by most patients with COPD. It is a distressing sensation which limits activity as it intensifies on even mild activity and exercise. There is evidence from several studies to suggest that the mechanism of dyspnoea may originate in lung receptors and be mediated by vagal afferent fibres to the brain where the sensation is perceived. There may be several concomitant mechanisms producing the sensation of dyspnoea including chemical, motor command and vagal. The evidence indicating a role for lung receptors being responsible as a pathway or component of this sensation is strong. The evidence

includes studies where symptomatic relief of dyspnoea was achieved by right surgical vagotomy in patients with emphysema, (Bradley, Hale, Pimble, Rowlandson and Noble, 1982), (Guz, Noble, Widdicombe, Trenchard, Mushin and Makey, 1966). When the only nervous communication between the thorax and the brain is via the vagi, as was the case in patients with transection of the spinal cord below the level of C3 and complete denervation below this level, the sensation of chest tightness can still be experienced, (Howell, 1969). In such studies the sensation of chest tightness could only be mediated by vagal afferent information.

Treatment of dyspnoea.

Experimental treatment of dyspnoea with various different types of tranquillisers has been studied, although it remains to be determined whether any psychotropic drug has a role in treatment of dyspnoea in COPD sufferers, (Light, 1991). There appears to be no role for chronic administration of benzodiazepines or tricyclic antidepressants, as neither give any alleviation of dyspnoea or improved exercise tolerance, (Light, 1991). The acute administration of opiates to patients with COPD gave significant improvements in their exercise tolerance, probably by decreasing the degree of discomfort for a given level of ventilation. However the effectiveness of this therapy on a long-term basis would carry great concerns about tolerance and addiction, (Light, 1991).

Limitations of exercise.

There is a large variation in the exercise tolerance of patients with chronic obstructive pulmonary disease who have a given level of pulmonary dysfunction and there is a relatively poor correlation between the exercise tolerance of patients with COPD and their level of pulmonary function, (Jones and Killian, 1991).

ANIMAL MODELS OF EMPHYSEMA.

To directly record pulmonary mechanoreceptor activity in emphysema it is necessary to have an animal model of the disease. A number of models were available for consideration.

Animal models of emphysema date back over a century, the early crude attempts at the induction of emphysema reflect the limited and poor understanding of emphysema at that time, (Snider, 1992). Little was known of the mechanisms involved in the production of the disease and most early models tried to produce emphysema by obstruction of the airways for example, as it was thought that the disease was due to a blockage of airflow. Development of acceptable models of emphysema awaited the significant recognition that enzyme-induced damage to pulmonary connective tissue components was of critical importance in the destruction of alveolar walls. Proteolytic enzymes such as papain, (Gross, Babyak, Tolker and Kaschak, 1964) and later elastase, (Kaplan, Kuhn, Pierce, 1973) were used to produce lesions in the lungs that more closely approximated the morphologic and physiologic features of emphysema. Although advances have been made, models of emphysema still bear little resemblance to the actual disease in terms of pathogenesis and must be viewed as analogues rather than equivalents of the human disease.

Several models of emphysema have been used for experimental investigations. These include papain-induced emphysema, Elastase-induced emphysema, emphysema induced by endogenous proteases such as cadmium chloride/ β -aminopropionitrile, nitrogen dioxide, and endotoxin, emphysema produced by impaired elastogenesis including copper deficiency, emphysema in the blotchy mouse, and models of uncertain pathogenesis involving starvation and the emphysema observed in the "Tight-skin" mouse.

EMPHYSEMA PRODUCED BY EXOGENOUS PROTEASES

Papain - induced emphysema.

The use of papain for the induction of emphysema was a breakthrough in the development of animal models of the disease, (Gross, Babyak, Tolker and Kaschak, 1964). The use of papain was originally intended as a possible treatment of pulmonary fibrosis, however intratracheal administration of papain solutions produced prominent airspace enlargement similar to panulobular emphysema.

Air space enlargement is accompanied by increases in total lung capacity, functional residual capacity, and compliance, (Pushpakom, Hogg, Woolcock, *et al.* 1970). This injury is also associated with damage to lung elastic fibres, which remain disrupted despite undergoing resynthesis, (Goldring, Greenburg and Ratner, 1968). Consequently, there is no restoration of lung recoil.

Elastase - induced emphysema.

Administration of pancreatic or leukocyte elastase into the lungs of animals causes rapid destruction of elastic fibres, resulting in diffuse airspace enlargement, (Cantor and Turino, 1991). Pancreatic elastase tends to produce more severe lesions than the neutrophil elastase, although this enzyme may initially cause greater haemorrhage and cell damage than pancreatic elastase, (Cantor and Turino, 1991). Physiologic alterations are similar to those produced with papain induced emphysema; increases in total lung capacity, functional residual capacity, and compliance are all observed, along with a reduction in arterial oxygen tension, (Snider, Lucey and Stone, 1986).

EMPHYSEMA PRODUCED BY ENDOGENOUS PROTEASES.

Cadmium chloride / β - Aminopropionitrile induced emphysema.

Instillation of cadmium chloride along with the cross link inhibitor, β -Aminopropionitrile (BAPN) results in an injury similar to emphysema, (Snider, Lucey and Stone, 1986). Cadmium chloride, initially induces an inflammation which causes the release of enzymes that degrade the matrix, then BAPN impairs the proper assembly of newly synthesised collagen and elastin, leading eventually to rupture of the alveolar walls. A substantial number of animals die from pneumothorax during the first month after administration of these substances. The animals that survive show increases in lung volume and compliance, (Cantor and Turino, 1991). Since this method of producing emphysema relies on endogenous proteases to cause lung

damage it may be more representative of the actual disease process than non endogenous protease administration.

Nitrogen dioxide - induced emphysema.

Prolonged inhalation of as little as 30 parts per million of nitrogen dioxide causes focal air space enlargement, (Kleinerman, J. 1979). The resulting lesions are confined mostly to the terminal bronchioles and alveolar ducts and therefore resemble centriacinar emphysema. Elastases released from neutrophils in the lungs are thought to responsible for the production of the disease, but it is also suggested that nitrogen dioxide may also interfere with elastic fibre repair, (Cantor and Turino, 1991).

Endotoxin - induced emphysema.

Endotoxin administration initiates inflammatory reaction that disrupts the pulmonary matrix. Repeated infusion of the material is necessary and even then only a small increase in air space size is produced, (Cantor and Turino, 1991).

EMPHYSEMA PRODUCED BY IMPAIRED ELASTOGENESIS.

Copper deficiency - induced emphysema.

A restriction of copper intake during lung development can induce emphysema, (Snider, Lucey and Stone, 1986). The disease is thought to be due to a decrease in the antioxidant protection mechanism of the lungs, (Snider, Lucey and Stone, 1986).

Emphysema in the blotchy mouse.

The blotchy mouse is a genetic model of altered copper transport in which diffuse air space enlargement of the panulobular type occurs spontaneously, (Snider, Lucey and Stone, 1986). Increases in the size of the airspaces are believed to result from a genetic loss of lysyl oxidase activity, although abnormality in macrophage function may contribute to the development of emphysema, (Cantor and Turino, 1991). The emphysema is seen very shortly after birth, and becomes progressively worse as the animal matures, (Cantor and Turino, 1991).

MODELS OF UNCERTAIN PATHOGENESIS.

Starvation - induced emphysema.

Marked loss of lung volume and weight can be caused as a result of starvation of new-born animals, (Cantor and Turino, 1991). Less severe damage is produced in the starvation of mature animals where the lung disease is characterised by an increase in alveolar size. The cause of this air space enlargement has not been determined but could result from an insufficient repair response to normal degradative processes. A similar type of lung abnormality is induced by restricting the intake of specific nutrients such as lysine, (Cantor and Turino, 1986).

Emphysema in the tight-skin mouse.

The tight-skin mouse is another genetic model of emphysema where the underlying biochemical abnormality is unclear, (Cantor and Turino). The emphysema is thought to result from elastases produced from increased numbers of inflammatory cells found in the lungs of these animals, (Snider, Lucey and Stone, 1986). Alveolar dilation develops 6 to 8 weeks after birth and is associated with increase in both lung volume and compliance, (Cantor and Turino, 1991).

Spontaneously occurring emphysema in animals.

Spontaneously occurring emphysema has been reported in several species of animals including cattle, horses, rabbits, dogs and rats, (Karlinsky and Snider, 1978). The acute pulmonary emphysema in cattle is of an unknown origin and does not appear to resemble human emphysema. Some horses with heaves, a respiratory disease of more chronic nature, show pathological changes bearing close similarity to human centrilobular and panlobular emphysema.

Spontaneous emphysema in rats is associated with murine pneumonitis and may be a paracatricial form of the disease, (Karlinsky and Snider, 1978). Nearly a third of all rabbits over 2.5 years old will have emphysema, some of this disease being associated with chronic interstitial fibrosis, (Karlinsky and Snider, 1978).

SELECTION OF AN ANIMAL MODEL FOR THE PRESENT STUDY.

Human emphysema can be appropriately related to animal models of emphysema, (Snider, Lucey and Stone, 1986). However the model chosen for a study can only be used to answer a limited number of scientific questions and because of this *"the usefulness of an experimental model should be judged on how well it answers the specific questions it is being used to answer rather than on how well it mimics human disease"*, (Snider, Lucey and Stone, 1986). A number of animal models of emphysema were considered for this study. The main consideration was to choose an appropriate animal model for the investigation into the breathing pattern and receptor activity in emphysema.

EVOLUTION OF THE LESION PRODUCED BY PAPAIN AND PANCREATIC ELASTASE.

The direct intrapulmonary induction of emphysema is a common method used to produce emphysema in the rodent. In the hamster, after a single intratracheal dose of porcine pancreatic elastase, respiratory air-space enlargement is noted within hours (Snider, Lucey and Stone, 1986). A similar time course of development of emphysematous lesions to that in the hamsters is observed in rats treated with a single intratracheal administration of papain, (Gross, Pfitzer, Tolker, Babjak and Kaschak, 1965).

Within a matter of minutes of pancreatic elastase enzyme being administered in hamsters, there is a change in the surfactant activity and the elastase begins to pass into the alveolar type I epithelium, (Snider, Lucey and Stone, 1986). Once inside the interstitium of the lungs the enzyme quickly spreads and attacks the elastic fibres. Elastase entering the blood is inactivated and cleared from the lungs. Other portions of the elastase are cleared more slowly by the macrophages, (Snider, Lucey and Stone, 1986). Degradation of elastic fibres started within fifteen minutes of the enzyme being instilled, (Snider, Lucey and Stone, 1986). The destruction progressed quickly with debris, possibly from ruptured alveolar walls, being observed after four hours, (Snider, Lucey and Stone, 1986). Synthesis of new elastic fibres began four days after exposure, as shown by the presence of small microfibrils clusters closely associated with interstitial cells, fibroblasts, and smooth muscle cells. The reformation of elastin fibres continued to take place over the following twenty days, but air-space size remains enlarged, (Snider, Lucey and Stone, 1986).

In rats emphysematous lesions develop within eight hours of elastase administration. The morphogenesis of the lesions is very similar to that of the other animals. The type I epithelium cells of the alveoli are damaged first before the elastin fibres are attacked by the enzyme, (Busch, Lauhala, Loscutoff and McDonald, 1984).

In rats that had been allowed to survive 2-3 weeks after instillation of elastase, changes in lung architecture were similar to those seen in rats killed at 1 week, (Busch Lauhala, Loscutoff and McDonald, 1984).

STRUCTURE AND FUNCTION OF THE LUNG IN RELATION TO THE SELECTED MODEL.

ANATOMY.

The mammalian lung exists primarily to permit gaseous exchange to occur. To perform this most vital function the lung possesses numerous thin-walled distensible air sacs connected by a series of passages to the external atmosphere. All mammalian lungs are constructed on a similar pattern although detailed structure may vary from species to species, (Krahl, 1964). The nasal passages and mouth communicate directly to the atmosphere: these lead into the cartilaginous trachea and this then divides at the carina to form the left and right main bronchi, which subsequently divide themselves and penetrate into the lung. The lung itself has two sides, the right and the left, which are usually divided into lobes. The number of lobes on each side of the lung differs between species, (Tyler, 1983). In man there are normally three lobes on the right and two on the left, (Tyler, 1983). In the rat there are four lobes on the right but the only one lobe on the left, (Tyler, 1983), as shown in Figure 1-3.

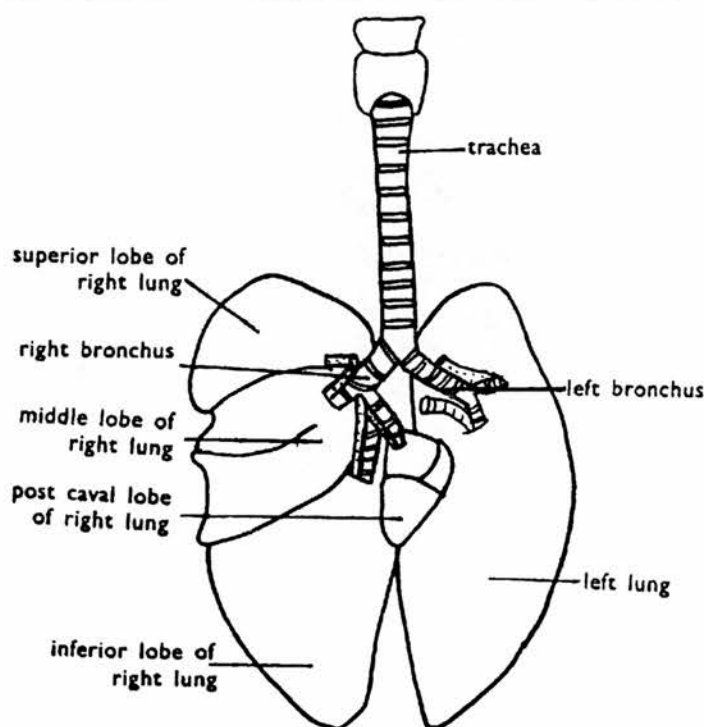


Figure 1-3: *Ventral view of the lungs of the rat.*

In the rat the right bronchus further divides outside the lung parenchyma and each of these branches enters one of the four separate lobes of the right lung. The left lung of the rat only has a single lobe and the left main bronchus divides into two branches immediately upon entering the lung.

On entering the lung the bronchi of the rat divide repeatedly into smaller and smaller diameter tubes, ultimately ending in the terminal bronchioles, (Tyler, 1983). In man these give rise to a further one to three generations of tubes known as respiratory bronchioles. In the rat, however, there are only a few respiratory bronchioles, (Tyler, 1983), and it is the terminal bronchioles and not the respiratory bronchioles which end in the alveolar ducts, (Tyler, 1983). The alveolar ducts terminate in two or more air sacs, and from these arise the alveoli which increase the surface area and constitute the gaseous exchange area.

The trachea and main bronchi are supported by a series of rings of cartilage, which in man extend along the intrapulmonary airways as far as the end of the small bronchi. In small mammals such as the rat, rings of cartilage only occur in the extrapulmonary airways and terminate in the hilum, (Krahl, 1964), see Figure 1-4.

The manner in which the airways divide to form a branching network is not the same in all species. In man it is a mixture of monopodal, polypodal and dichotomous branching. In the rat, because the left lung is a single lobe the way in which the airways branch is quite different (see Figure 1-4).

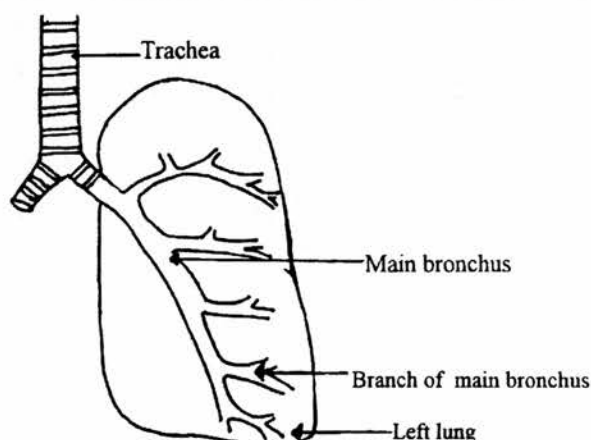


Figure 1-4: *Schematic diagram of rat lung, (left lobe) showing the left main bronchus and branches.*

Lung Parenchyma.

Lung parenchyma is composed of collagen, elastin, glycosaminoglycans and proteoglycans and fibronectin macromolecules, (Bruce, 1991). The elastic properties of the pulmonary parenchyma and vasculature are largely attributed to the presence of elastic fibres in the extracellular matrix. Elastic fibres account for approximately 25% of the connective tissue mass of the lung, and are an important component of alveolar walls, blood vessels and pleura, (Bruce, 1991). They have the ability to undergo expansion and return to their original configuration, (Bruce, 1991). The elastic fibres influence the efficiency of air movement through the lung. Loss of elastic fibres causes distension and rupture of the alveoli and may lead to severe respiratory dysfunction.

Lung Compliance

Lung compliance is a measure of elastic recoil. Due to destruction of lung tissue and loss of elastic fibres emphysematous lungs have a higher compliance, that is to produce a given volume there is a smaller pressure change. The highly extensible elastic fibres in the lung account for the steep slope of the volume/pressure curve in its mid-volume range, (Karlinsky and Snider, 1978). Elastolytic enzymes diminish tissue

recoil and increase compliance at mid-volume ranges as they destroy the elastic network of fibres. Increased lung compliance is seen in human emphysema.

PRESENT STUDY.

Animal models of emphysema have been used to investigate a variety of aspects of the disease. They have provided an important tool for investigating the various mechanisms of injury by mechanical stress, free radical, endogenous elastases, and inhibition of elastic fibre cross-linking. But as yet animal models of emphysema have not been used to quantify the changes in individual pulmonary receptor activity which are possibly the origin of the disordered patterns of breathing and perhaps, though more speculatively, the dyspnoea seen in the human disease.

AIM OF THIS THESIS.

In this study I investigate pattern of breathing, respiratory reflexes and the activity of vagal lung receptors in an animal model of emphysema. I relate changes in the pattern of receptor activity produced in the emphysematous model to changes in the pattern of breathing and to alterations in the reflex responses to inflation and deflation of the lungs.

Other workers have used blocking techniques or recorded bulk activity from vagal receptors in diseased models produced by acute injury, (Armstrong and Miller, 1980), (Frankstein and Sergeeva, 1966). However this study has recorded the type and degree of activity of individual receptors, as it was thought that this would give a more accurate and useful idea of the effect of individual types of receptor in the control of breathing in the diseased state. Activity from individual stretch and rapidly adapting receptors was recorded to determine whether changes in the activity from these receptors could possibly account for the changes in breathing pattern associated with emphysema and to relate any altered activity to dyspnoea, since removal of the influence of vagal lung receptors by vagotomy or vagal block gives relief from this sensation, (Guz, Noble, Eisele and Trenchard, 1969).

The specific aim was to investigate if changes in pulmonary receptor activity are produced in the model, and if these can be related to the disordered pattern of breathing and possibly dyspnoea in human emphysema.

CHAPTER 2

METHODS

The methods are divided into two sections:- A) Experimental Methods and B) Protocol.

SECTION A:- EXPERIMENTAL METHODS

INDUCTION OF EMPHYSEMA

It was decided to induce emphysema with the enzyme Papain, it was therefore necessary to have an estimation of the proteolytic activity of the Papain bought from the suppliers.

ESTIMATING THE ELASTOLYTIC ACTIVITY OF PAPAIN

Since the proteolytic enzyme Papain is a natural product extracted from the fruit and latex of the papaya tree, different batches of Papain contain slightly varying amounts of the active enzymes. A sufficient quantity of Papain was therefore obtained in a single batch at the beginning of the study for all the experiments. By homogenising this single sample the dose given to each animal would be roughly equivalent. An estimation of the proteolytic activity of the crude Papain, from the suppliers, was necessary so that doses, comparable with those in the literature, could be administered. To obtain an estimate of the elastolytic activity of the crude Papain the following assay was employed.

A modification of the Congo red-elastin assay of Naughton and Sanger, (Naughton and Sanger, 1961) was used out to determine the elastolytic activity of the crude Papain, (Sigma, UK). The time taken for the Papain to digest the substrate, (elastin) to a defined stage of digestion, namely 50% solubilisation was measured. This time bears a close inverse ratio to the amount of elastase present.

Congo red -elastin.

Congo red-elastin E-0502, (Sigma, UK.) is made from bovine ligamentum nuchae elastin free from collagen and other contaminants, stained with Congo red. The Congo red-elastin is supplied in a finely ground form, and was suspended in 0.05M phosphate buffer, pH 6.5, at a concentration of 1mg/ml.

Papain

Papain was ground into a powder in a mortar and activator solution added. Three concentrations of Papain were assayed.

- 1.0 mg of Papain /ml of activator solution.
- 10 mg of Papain /ml of activator solution.
- 100 mg of Papain / ml of activator solution.

Activator solution for Papain.

- 5.5×10^{-3} M L-Cysteine
- 1.1×10^{-3} M EDTA
- 100 ml phosphate buffer
- 1 drop of mercaptoethanol/100 ml.

PROCEDURE.

1. 70 ml of phosphate buffer, pH 6.5 was placed in a beaker and 0.07g of Congo red-elastin was added.
2. Papain was weighed out. In each of seven plastic tubes known weights of ground Papain were placed: for the first assay 1mg of Papain was used, in the second 10mg was used and in the third 100mg was used.
3. At intervals each of the tubes containing Papain had 7 ml of Congo red-elastin solution, in the phosphate buffer, and 1ml of activator solution added. Timing was started. The tubes were stoppered and shaken vigorously for 5 seconds.
4. The tubes containing the Papain, Congo red-elastin, activator solution and buffer were placed in a water bath at 37°C and stirred continuously.
5. At given time intervals the contents of the tubes were mixed, centrifuged for 30 seconds, and the optical density of the supernatant measured at 495nm in a spectrophotometer, (Pye unicam SP6-550 UV/VIS).
6. In another tube, tube 8, 1ml of activator solution was added to 7ml of Congo red-elastin but no Papain was put in.
7. The optical density of the solution in tube 8 was measured against a blank of distilled water at 495nm in a spectrophotometer.

The assay was carried out in triplicate. There were three tubes setup for each time interval at which the optical density of the supernatant was measured. So in total 21, (7x3) tubes containing Papain, buffered Congo red-elastin and activator solution were prepared for each of the three concentrations of Papain solutions assayed.

Six elastase assays were performed to produce a standard curve from which equivalent concentrations of elastase activity could be found for certain weights of Papain.

From the smooth sigmoidal progress curve obtained for the assay by plotting the increase in optical density against time, the time taken to achieve 50% of the Congo red into solution is determined. A plot of such times obtained for a series of calibration assays against the reciprocal of pure elastase present gives a calibration curve which is almost linear, from which the amount of elastolytic activity of the solutions being assayed can be determined.

METHOD OF EMPHYSEMA INDUCTION

The rats were induced with emphysema by endotracheal administration of Papain.

PRELIMINARY DOSING

Preliminary tests were carried out to find a suitable dose of crude Papain with which to treat the rats. For the study into pattern of breathing and lung receptor activity in emphysema it was necessary to produce pathological statistically significant increases in the mean linear intercept values of the alveoli of the papain treated, compared to the control rats. In the preliminary tests a group of rats were treated with varying doses of papain. Each rat received, by insufflation, a single dose of either 1, 2, 4, 8 or 12mg of Papain/100g of body weight. The volume of saline solution in which the papain was suspended was also decided on at this stage. The smallest volume possible of the solution was desirable, but it had to be sufficiently fluid to carry the papain to the lungs without becoming blocked in the tracheal catheter. The rats were carefully and regularly observed after their papain administration. Any signs of distress were noted. Any animal observed to be in distress was immediately killed by an overdose of anaesthetic. Those rats showing no distress were left to recover for 1 or 4 weeks, killed with an overdose of anaesthetic and their lungs removed for morphometric analysis. The lungs were histologically prepared and examined for evidence of emphysema.

INSUFFLATION

Endotracheal administration of papain took place with the rats under anaesthesia. The rats were anaesthetised with 4% Halothane in Oxygen in a sealed box connected via a thick walled rubber tubing to a Boyle Fluotec3 Anaesthesia Machine, (Cyprane LTD, UK).

The animals were then held in a vertical position by the scruff of their necks and their mouth kept open with the tongue pulled to one side. The glottis was visualised with the aid of a fibre optic light source which provided a strong direct light and a small metal spatula that was used to depress the tongue. A plastic endotracheal tube made from a pink luer catheter, (Portex, France) cut to 16cm in length, containing a wire, as an introducer, was inserted gently down the trachea for 10cm so that the end of the catheter was at the carina of the lungs. The introducing wire was then removed

from the catheter and a dose 0.05ml/100g body weight of a 240mg/ml solution of papain in normal saline was injected through the catheter. Crude papain was used as purified preparations of papain have elastolytic activity removed during the purification process, (Snider, Hayes, Franzblau, Kagan, Stone and Korthy, 1974). The volume administered for a 500g rat was therefore 0.25ml of drug solution containing a total of 60mg of papain. The papain solution was administered carefully to ensure the full dose was given. An additional 0.05 ml of papain solution was added to the dose to take account of the calculated dead space of the catheter. The catheter was then removed. The rat was then tilted up and down to ensure that the papain solution reached both upper and lower regions of the lungs.

The rats were then left to recover from the anaesthetic. They usually recovered within 5 minutes of the end of the procedure. The condition of the animal was noted at this stage as to whether the animal was wheezing and if the sound of fluid could be heard on the lungs.

AEROSOL.

In case the insufflation method of inducing emphysema was unsuccessful I prepared to produce emphysema by an aerosol technique. For this method of inducing emphysema an aerosol generator of novel design was constructed. This is included in the thesis as it might be of interest for use in other studies.

Two major types of generator in common use for the production of aerosols are *jet*, driven by compressed air, and the *ultrasonic* type which relies on a driven element vibrating at ultrasonic frequency in the liquid to be aerosolised. The output characteristics of these devices differ in terms of their rates of nebulization and the size and range of the droplets produced, (Hardy, Newman and Knoch, 1993).

Ultrasonic generators offer many advantages over jet types. They produce a dense cloud and therefore can deliver a high dose in a short time. The distribution of droplet size they produce, median diameter 5.2-6.9 μm , (Lourenco and Cotromanes, 1982) and therefore the site of deposition in the lungs, is narrower. Their main disadvantages are that they are expensive compared to other types, and usually require a relatively large charge of liquid being aerosolised.

For this study an aerosol generator was required that could produce and deposit small droplets of Papain deep into the lungs of the rats in as short a time as possible, to minimise distress to the animal. So it was decided to construct an ultrasonic nebulizer as this type of generator usually produces a dense cloud of small droplets, which penetrate the periphery of the lungs and deliver the drug quickly.

To overcome the problems of expense and the usual requirement of a large charge of liquid to be aerosolised a "Pifco" Domestic Humidifier (Pifco Ltd.,

Sailsworth, Manchester, M35 OHS) was modified to aerosolise small (down to 5ml) samples of liquid or papain solution which could then be administered to the rats.

A diagram of the ultrasonic aerosol generator is shown in Figure 2-1. The original humidifier consisted of those parts shown (not cross-hatched) plus a simple plastic box containing a water bottle, which provided a constant level of water over the ultrasonic transducer. The box also prevented large particles splashing out of the system. The box and water bottle were discarded for the new instrument.

In its original form the ultrasonic transducer dissipated its energy throughout the surface of the water placed in the humidifier. In the modified instrument the energy is focused by a tubular energy-guide on to the base of the sample tube. The lower end of the tube consists of a thin rubber diaphragm, made of a piece of a child's balloon tied in place with thread. This was immersed in the coupling-water which covers the transducer. The parts that have been added to the original humidifier are shown in cross-hatched section and are constructed from perspex.

An air-supply, part of the original humidifier, consists of a small fan which provides a controllable stream of low pressure air. This is contained within the air-box and admitted to the sample-tube, and up another tube which prevents the escape of large drops of liquid and returns them to the body of the sample.

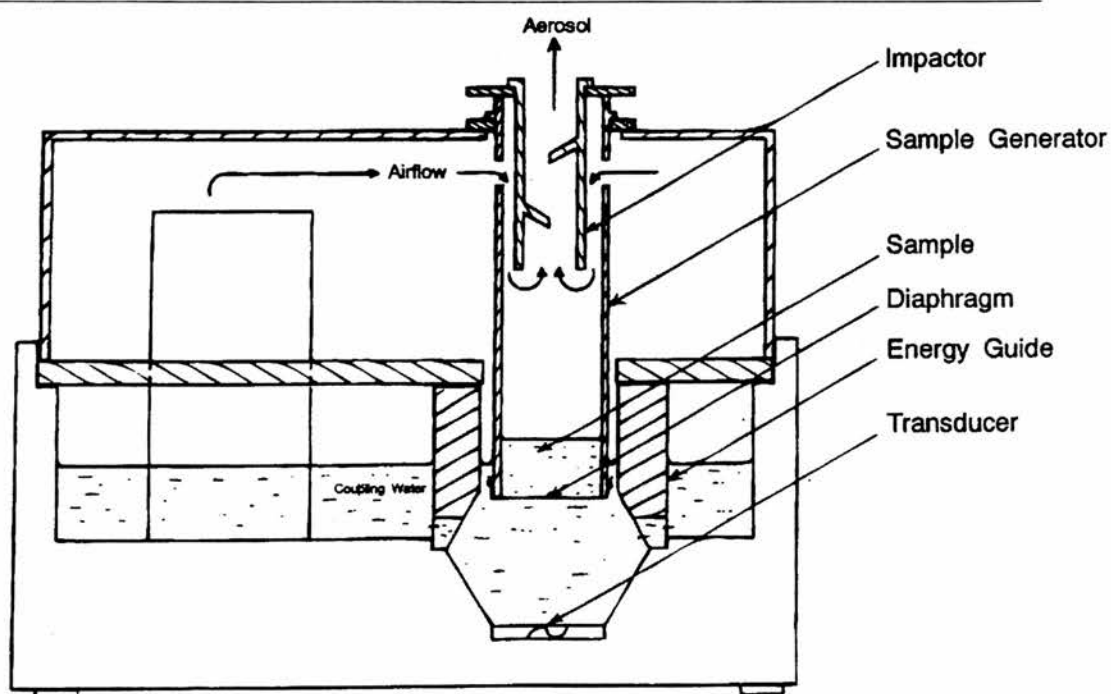


Figure 2-1: *Vertical section of an inexpensive aerosol generator modified from a domestic humidifier.*

It was necessary to characterise the aerosol produced since particle size distribution and density of the aerosol, that is the amount of liquid suspended in a given volume of air, are important determinants in the efficacy of the drug delivered. These characteristics were determined by passing it, immediately after it left the generator, through the beam of a "Malvern 2600" laser defraction droplet size analyser, (Malvern Instruments, Spring Lane, Malvern, WR1 1AQ). In this instrument the sample is illuminated by a 2mW, 632.8nm helium-neon laser. The particles scatter the light at angles which are characteristic of their size, forming a series of diffraction patterns. The scattered light is collected by a Fourier optical system and focused on a detector. Here the light is amplified, digitised and the complete light energy pattern is then analysed by computer (see Figure 2-2).

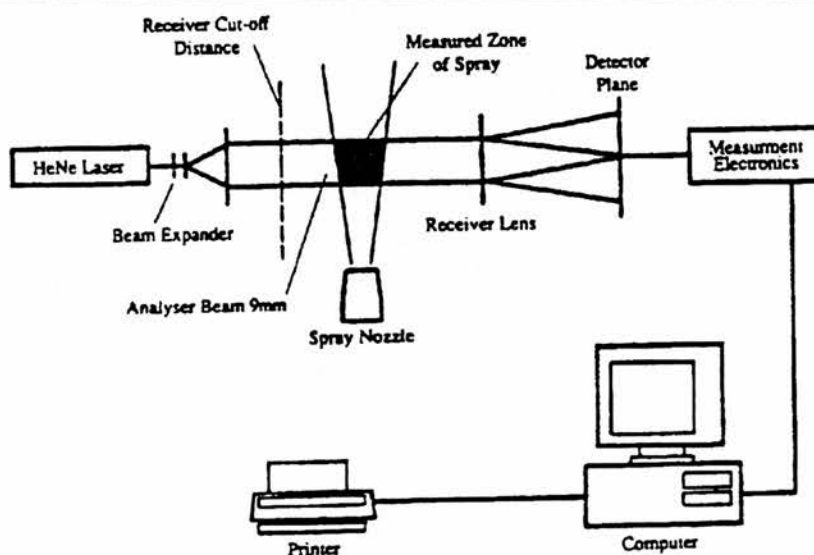


Figure 2-2: Setup of the Malvern aerosol particle sizer equipment.

QUANTIFICATION OF EMPHYSEMA

SAMPLING OF THE LUNG.

In any histological analysis of the lung, sampling is all important. In the normal lung few problems in sampling occur as apart from a small region near the hilum, the parenchyma is fairly uniform. However in lung disease this uniformity may well be absent. The most accurate assessment of any lung disease would involve examining the whole of the lung, but this is very time consuming, particularly in large human lungs. A balance must therefore be struck between accuracy of assessment and practicality. Two methods of sampling could be employed.

1. Systematic sampling, where samples are taken at fixed areas or at given intervals of the lung. The main disadvantage is that if the disease displays a non uniform arrangement similar to the sampling pattern, the sample will be unrepresentative of the lung as a whole, and therefore any results could give a false impression of the real state of the disease in the organ.

2. Random sampling, where the selection of blocks is made using a random number table. In theory this would produce blocks which possess the same characteristics as the entire lung, however in practice a highly unrepresentative sample could be selected, i.e. if two adjacent blocks in an lower lobe were taken and none from the upper in a given slice.

To prevent a biased selection of blocks occurring a combination of systematic and random sampling can be used known as stratified random sampling which combines the advantages of random sampling yet imposes some restrictions on the samples selected, (Dunhill, 1962). This is method is particularly useful for sampling large lungs such as humans where it is often not practical to examine the entire lung. In lungs from small animals it is often feasible to examine the whole, or a large portion of the lung reducing the need for sampling.

I decided to sample from the single lobed left lung of the rat. The reasons and justification for sampling from this side of the lung are that it could easily be divided as it is a single lobe; also the production of emphysema due to administration of intratracheal papain had an equal probability of occurring in either side of the lung and to a similar degree, so no particular bias was being introduced by using only the left side. The same sampling was used for both the controls and the treated animals. In this particular study it was not thought essential to sample the both sides of the lung, as the presence of emphysema if found in the left lobe would indicate that the disease had been induced in the animals, and this was adequate assessment for this

investigation. Since the most accurate form of assessing is to examine as large an area as possible I selected three 1cm by 1cm blocks cut from the left lung. This gave a sample of practically the whole length of the left lobe as the lung is only approximately 5cm in length, so stratified and random selection of blocks was not employed as practically the entire lobe was being sampled.

METHOD OF HISTOLOGICAL TECHNIQUES EMPLOYED IN THE QUANTIFICATION OF EMPHYSEMA.

For details of all fixatives and reagents used for histological purposes in this study see Appendix 2.

LUNG FIXATION

The lungs were dissected from the animal and inflated as follows:

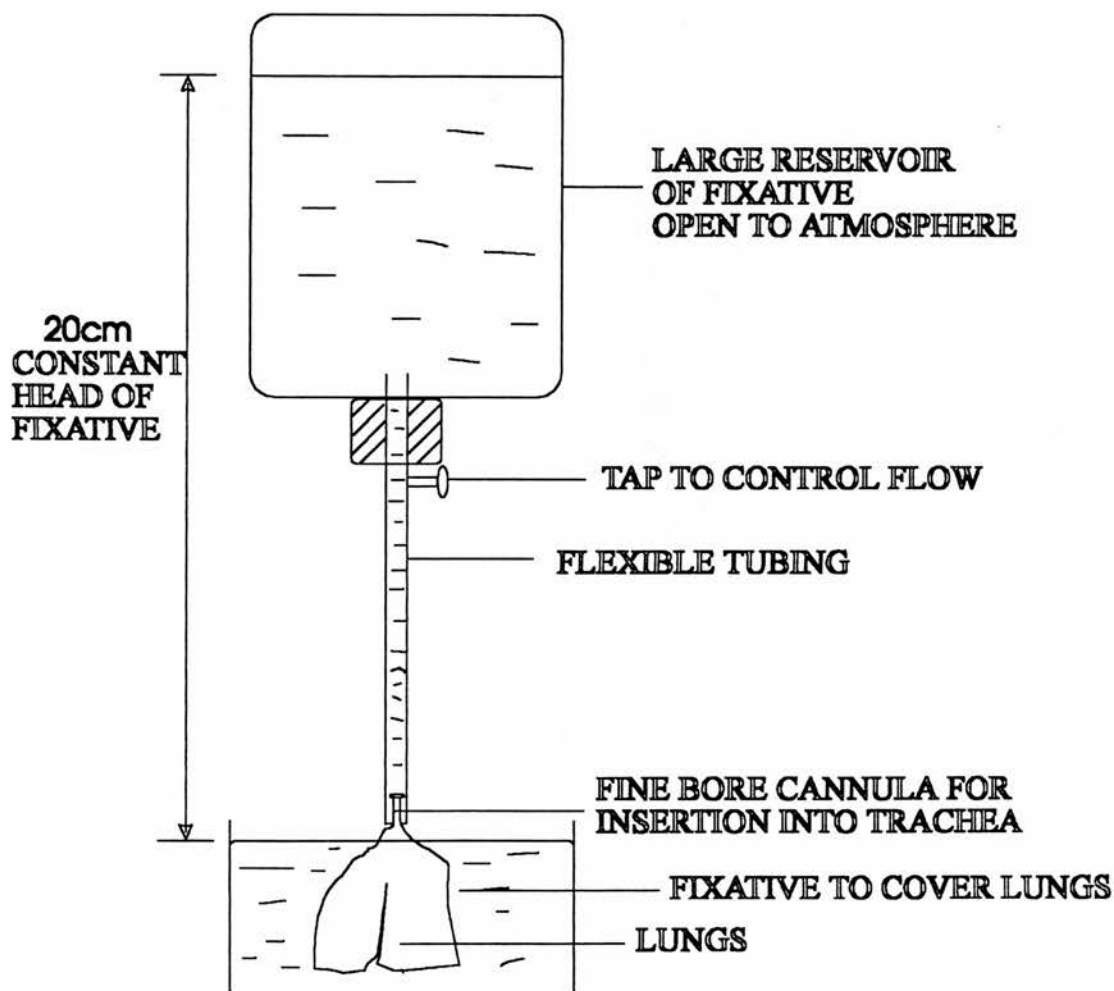


Figure 2.3: *The experimental apparatus for inflation-fixation of rat lungs.*

A diagram of the apparatus is shown in Figure 2.3. The apparatus was designed so that the fixative was delivered at a constant pressure. This was achieved by having a very large reservoir of fixative at 20 cms above the lungs. The reservoir was connected to the lungs by a plastic tube with a 3-way tap, at the end of which a small plastic cannula was attached. The cannula was inserted inside the trachea and tied securely in place with thread so as to prevent any leakage. As soon as the tap was opened fixative flowed down into the lungs from the reservoir. When the lungs were fully inflated back pressure stopped the flow of fixative, the lungs were left to inflate at this constant pressure for 24 hours. After this time the tracheal cannula was removed and the trachea tied tight. The lungs were then placed in a specimen jar and covered with fresh formal saline until they were processed further.

TECHNIQUE OF TRIMMING AND PREPARING BLOCKS OF LUNG FOR EMBEDDING IN PLASTIC.

The left lung was used for the microscopic assessment of emphysema. The whole lungs were placed on a board, ventral surface uppermost. The lateral surface of the left lung was taken and using a sharp dermatone blade a 0.5cm thick section was trimmed from the lateral surface with a single stroke of the knife. Sawing-like action was avoided so as not to crush the tissue. Next a 1cm section was cut from the cut edge of the lung, and 1cm by 1cm blocks of lung were cut from this mid-sagittal section of the left lung. Three blocks were cut from each mid-sagittal section an upper, middle and lower block. The blocks were then placed in labelled 50ml specimen pots ready for dehydration through graded alcohols.

PROCESSING OF FIXED TISSUE IN GLYCOLMETHACRYLATE FOR MICROSCOPIC EMPHYSEMA ASSESSMENT.

The fixed and trimmed blocks were dehydrated through a series of alcohols over several days at room temperature. Firstly the blocks were placed in a 10% alcohol solution and left to dehydrate for some time, then the alcohol was poured off and replaced with fresh 10% alcohol solution and the tissue left to dehydrate further. This procedure was repeated putting the blocks in 30% alcohol solution, 50% alcohol, then 60%, 70%, 80%, 90%, 95% alcohol and finally in absolute alcohol. The blocks remained at each stage for at least two hours, and had numerous changes of alcohol solutions to ensure complete dehydration, as the tissue blocks were large and therefore required a long time for the alcohol to dehydrate them.

IMPREGNATION AND EMBEDDING OF TISSUE IN PLASTIC RESIN.

METHOD FOR EMBEDDING TISSUE IN GLYCOLMETHACRYLATE

All the solutions were made up in a fume cupboard.

Composition of solutions used.

GMA Solution A (impregnator solution)

80ml 2-hydroxyethyl methacrylate

8ml 2-butoxyethanol

0.5g benzoyl peroxide

(This solution was prepared immediately before use with the peroxide added last).

GMA Solution B (Promoter solution)

8ml polyethylene glycol 400

1ml N.N.dimethylaniline

(Solution B was also prepared immediately before use).

GMA polymerising solution

42 parts solution A

1 part solution B

(The solution was mixed thoroughly and again used immediately).

TECHNIQUE OF IMPREGNATING THE LUNG TISSUE WITH PLASTIC.

The embedding procedure involving the polymerisation reaction was carried out in the fumehood.

1. Tissue was removed from the absolute alcohol in which it had been dehydrating and placed in a clean container. Sufficient Solution A was then put into the container to cover the tissue. The container was then placed in vacuum chamber under a pressure of 27 inches of Hg below atmospheric. The tissue was left under this vacuum at room temperature for 24 hours.

2. After 24 hours the tissue was put in GMA solution A and again left for at least 24 hours under vacuum. The tissue was changed once more into fresh solution A and left for another 24 hours under vacuum.

3. For each batch of lungs that were embedded a test polymerisation reaction was carried out to check that the polymerisation occurred controllably. If the reaction

occurs too quickly there is not enough time to cool the reaction and to prevent bubbles forming in the plastic, causing damage, by excessive heat, to the tissue.

4. Sufficient solution for the whole batch to be processed was then made up.

5. The tissue was placed in plastic embedding moulds (Polysciences INC, Warrington, UK.), with the lateral surface placed at the bottom of the mould. The polymerisation solution was poured over them to 3/4 fill the moulds. With a pair of round ended plastic forceps the tissue was squeezed gently and pushed to the bottom of the mould to make sure all the air was removed from the lungs. The polymerisation usually took about 10-15 minutes before the solution started to become tacky and thicken depending on the temperature of the surroundings.

6. Once the reaction was under way and heat was being produced the moulds were transferred on to ice to dissipate the heat and prevent gas bubbles forming in the blocks.

7. When the blocks became hard they were placed in a moderate 60°C oven overnight to fully harden and then removed from the moulds.

8. Blocks were trimmed with a hacksaw to a convenient size for cutting.

PRODUCTION OF THIN PLASTIC LUNG SECTIONS.

All the blocks were trimmed and orientated using a Reichert-Jung Autocut Microtome. A tungsten-carbide coated knife was used on the microtome to cut the blocks into 6µm plastic glycolmethacrylate sections. Thick sections were cut from the block until lung tissue started to be appear in the sections. Trimming continued until the block being cut produced a complete 1cm x 1cm section of lung. The microtome was then set to cut at 3µm and several sections were cut and collected. These sections were floated out on a warm water bath and individually guided on to gelatine coated microscope slides. The end of the slides were held below the surface of the water in the bath and the section carefully floated on to the wet slides. The slide was taken out of the water and left to dry, usually overnight. After drying the sections were stained in Bullard's Haematoxylin and Eosin.

METHOD OF STAINING THE SLIDES IN HAEMATOXYLIN AND EOSIN:

The Bullard's haematoxylin was first made up with the ingredients listed below:-

Bullard's Haematoxylin:

8g haematoxylin
16ml glacial acetic acid
144ml 50% ethanol
20g aluminium ammonium sulphate
250ml distilled water
8g red mercuric oxide
275ml 95% ethanol
330ml glycerol
18ml glacial acetic acid
40g aluminium ammonium sulphate.

Preparation of Haematoxylin stain

Then the haematoxylin stain was prepared. The haematoxylin was dissolved in the 144 ml of 50% ethanol. 16 ml of glacial acetic acid was added. This mixture was put into a heated solution of 20 mg aluminium ammonium sulphate dissolved in 250 ml H₂O. The mixture was heated to boiling and the 8g of red mercuric oxide was slowly added (to prevent frothing). The resulting mixture was then cooled rapidly and filtered. Then 275 ml of 95% ethanol, 330 ml of glycerol, 18 ml of glacial acetic acid, and finally 40g of aluminium ammonium sulphate was added to the mixture.

Staining

The sections were stained in Bullard's haematoxylin at room temperature for 5 minutes and then briefly washed in water. They were then dipped in 1% acid alcohol and blued in Scott's tap water for 30-60 seconds. They were then counter stained in 1% eosin for 20-30 seconds. Sections were rinsed in water and dehydrated through graded alcohols to xylene prior to mounting.

Mounting Stained Sections

The slides had glass cover slips put over them which were mounted using DePeX.

METHOD OF MEAN LINEAR INTERCEPT ASSESSMENT OF EMPHYSEMA.

The Mean linear intercept, (average distance between alveolar walls), was measured for two sections from each block. The method used was similar to that of (Dunhill, 1962), where the stained lung section slides were examined under a microscope with a crossed hair line of equal and known length fitted to an eyepiece, (Dunhill, 1962). The two hair lines at right angles to each other compensate for deformation of the tissue due to cutting and mounting.

The sections were subsampled by a random procedure. This was achieved by blind random displacement of the mechanical stage of the microscope without observing through the tube. Random fields from the sections were viewed, ten fields per section being examined. For each field the number of points at which the lines on the cross hair cut through the alveolar walls were counted. A cut through an alveolar wall counts as a single intercept, a cut into a blood vessel wall counts as half an interval as does a cut out of a blood vessel wall. In this way the number of intercepts were counted on both the vertical and the horizontal hair line for each field, and the sum for all the fields on a section was obtained. The mean linear intercept L_m was then calculated from m , the sum of all the intercepts, L , the length of the transverses, and N , the number of times the transverses are placed on the lung where ($L_m = N.L./m$).

ANIMALS AND ANAESTHESIA

All rats used were supplied by B.K. Universal LTD. UK and were of the Sprague Dawley strain, weight range 477-663g. They were not bred in strictly pathogen free conditions but were free from overt respiratory disease. The animals were kept in a 12 hour light and dark cycle and fed ad libitum with standard rat diet and water. The rats were housed in the animal house after transfer from the suppliers and allowed at least one full week to acclimatise to their new surroundings before being used in an experiment. During this time they were observed and none of the animals used had any obvious signs of respiratory disease such as coughing, sneezing or nasal discharge prior to being used.

The rats weighed on average 579 \pm 21.4g (controls) and 625 \pm 20.6g (treated). They were initially anaesthetised with 3% Halothane in Oxygen in a sealed box, connected to a Boyle Fluotec3 Anaesthesia machine, (Cyprane LTD, UK.) as recommended by Flecknell, (Flecknell, 1991). The rats were then given an intraperitoneal dose of ethyl carbamate solution (Urethane, Sigma UK.), 25% in saline (0.6ml/100g). A surgical plane of anaesthesia was maintained throughout the experiments with periodic intraperitoneal injections of one quarter of the original dosage of the anaesthetic agent. The level of anaesthesia was determined by testing withdrawal reflex by pinching the hind paw of the rat.

ANIMAL PREPARATION

Five minutes after a surgical level of anaesthesia was produced the number of breaths taken in 1 minute by the rat was recorded. The rat was observed for chest movements for a period of one minute. The breathing rate was counted three times and the mean value found. This estimate of breathing frequency, by breath counting, was then repeated after tracheal cannulation of the rats before and after connection to the pneumotachograph.

The rat was placed in the supine position. A mid-line incision was made in the neck and the trachea was then located. The trachea was cut with a pair of fine scissors through one of the rings of cartilage and wiped free of any blood with a piece of cotton wool, to prevent any obstruction. A plastic tracheal cannula of a suitable size and diameter (2mm inside, 3mm outside diameter) for the rats trachea, with internal tracheal diameter of 3mm, was inserted and tied into the trachea of the animal. The cannula was inserted between the 2nd and 3rd cartilaginous ring below the larynx, sparing the recurrent laryngeal nerve.

INSTRUMENTATION AND PHYSIOLOGICAL MEASUREMENTS

Airflow was recorded by a Fleisch pneumotachograph head (Flowhead F10L, Mercury Electronics LTD, U.K.) connected to the tracheal cannula. Tidal volume was obtained by electronically integrating flow on a flow meter. Carbon dioxide in the inspired air was monitored by an infra-red gas analyser (Beckman L.B.1), sampling from the rostral side of the pneumotachograph head. A two minute period of control breathing was recorded.

The rat's responses to steps of maintained lung inflations or deflations (0.5 kPa, (5cm.H₂O)) and (1 kPa, (10cm.H₂O)) for several seconds were recorded. The inflation pressure was maintained until the animal took a voluntary inspiration or it was considered that the length of time elapsed was dangerous for the animal. Deflation pressure was maintained for a period of ten consecutive breaths from the onset of the pressure. The inflation or deflation of the lungs was achieved by connecting the tracheal cannula to a 20 litre drum of air held at positive or negative pressure by switching a solenoid valve. The pressure for the inflations was generated from the exhaust port of a vacuum motor, while the deflation pressure was generated by the suction from the vacuum motor. The inflation or deflation of the lungs was synchronised with the beginning of inspiration or expiration respectively.

Arterial partial pressure of carbon dioxide was increased by causing the animals to breathe approximately 4% and 6% carbon dioxide for 2 minutes. The carbon dioxide mixtures were prepared by mixing commercial carbon dioxide with air and passing the resulting mixture across the free end of the pneumotachograph head. The two minute period of breathing whilst the animals were inhaling the carbon dioxide was recorded at both the levels of CO₂.

RECORDING ARTERIAL BLOOD GASES.

With the rats in the supine position an incision in the left groin of the rat was made and the femoral artery located. A plastic catheter with a three way tap containing heparnised saline was inserted into the artery. The catheter was flushed with heparnised saline and arterial blood withdrawn into a syringe. A 0.5ml arterial blood sample was taken with a clean heparnised 1ml plastic syringe, which was immediately capped to prevent air contamination. The sample was kept under ice until analysis for oxygen and carbon dioxide gas tensions was performed using a Ciba-Corning Diagnostics LTD U.K. 238 pH / Blood Gas Analyser. The blood sample was analysed within a few minutes of being taken.

LUNG COMPLIANCE MEASUREMENTS.

Static lung compliance (Cst) with the lungs in situ was determined in 6 control and 6 emphysematous rats.

Following cessation of respiratory effort after overdose of anaesthetic the lungs were allowed to deflate and were left in the exhaled state at functional residual capacity (FRC). The lungs were initially actively inflated with a 10ml volume of room air contained in a syringe and then allowed to passively deflate to functional residual volume. This procedure was repeated twice to stabilise lung volume history prior to measurements of static compliance being made.

To measure Cst the lungs were inflated from FRC in a stepwise fashion, with 1ml volumes of air being injected at 1 minute intervals, until a total of 10ml of air had been injected into the lungs. Elastic recoil pressure of the lungs (Pst) were measured throughout the inflation. Outputs from the pressure measuring device were recorded on chart recorder paper. The Pst was related to the volume of air delivered, in order to determine the pressure-volume relationship of the lung.

By plotting volume injected against pressure a static inflation curve was obtained. The linear portion of the curve was determined and the slope of the curve at this point measured, this gave a value of Cst.

A deflation curve was obtained from the results of the pressure changes produced by withdrawing 1ml volumes of air from the lungs at 1 minute intervals, until a total of 10ml of air had been withdrawn.

NEUROPHYSIOLOGICAL MEASUREMENTS

The left vagus nerve was exposed high in the mid-cervical region of the neck. The nerve was carefully dissected from surrounding tissue using micro-dissecting forceps and scissors. The nerve was then cut as high as possible in the neck near the nodose ganglion to obtain a sufficient length of nerve to work with. The cut distal end was freed from surrounding tissue for about 5mm and placed in a copper tray containing liquid paraffin, to prevent drying of the nerve. Using micro-dissecting tools the nerve sheath was dissected away from the tip of the cut end, and thin fibre preparations were made from the nerve by stripping it apart with fine forceps. The thin fibre strands were placed on a pair of silver wire electrodes held by a micro-manipulator.

The signal from the electrodes was put through a high impedance unity gain preamplifier, (NL100 Headstage Neuralog System Digitimer LTD) and then amplified

on a high gain A.C. coupled differential amplifier, (NL104AC Preamplifier Neuralog System Digitimer LTD). This amplified the signal from between 100 to 1000 times. The signal was then filtered with an NL125 Filter, (Neuralog System Digitimer) to remove both high frequency and low frequency signals outside the appropriate bandwidth. The resulting amplified, filtered signal was displayed on an oscilloscope, (HM203-7 Hameg). The overall gain in the whole system was in the region of 10000. The signal was also audio amplified, (NL120, Neuralog System Digitimer LTD) so that the electrical activity of the nerve could be heard. The signal displayed on the oscilloscope was examined and if there was more than one receptor within the strand of nerve on the electrodes the strand was then divided again until only the action potentials from one receptor were observed. If the electrical activity from this fibre displayed respiratory rhythm it was then recorded, such a fibre was called a 'single fibre'.

In a preliminary study the conduction velocity of 12 fibres from 2 rats was measured by exposing as great a length of vagus as possible (usually about 10mm). A stimulating electrode was placed as far as possible from the recording electrodes and connected to a Neuralog stimulator. A supra-threshold stimulus applied to the nerve also triggered the sweep of an oscilloscope. Conduction velocity for a single fibre was calculated as the distance between stimulating and recording electrodes (measured with dividers) divided by the time from the stimulus artefact to the initial rise of the action potential of the fibre in question. It soon became apparent that conduction velocity measurements were unnecessary to differentiate between slowly and rapidly adapting receptors.

The fibres were classified as slowly adapting stretch, or rapidly adapting according to their response to a step of inflation of the lung by a pressure of 1 kPa (10cm.H₂O). The criterion which was set for a fibre being rapidly adapting was a fibre which was silenced within 0.25 seconds from the onset of the inflation, while fibres which continued to fire during this period of inflation were classified as slowly adapting. This was sufficient to clearly distinguish between the two types of receptors.

The raw electrical activity of the fibre preparations was then recorded together with the other physiological variables of flow, volume, and carbon dioxide, on magnetic tape by a 5 channel TEAC XR-30 recorder (TEAC, Japan). The raw electrical activity from the electrodes was also displayed on an oscilloscope and the output from this was separately relayed to a spike trigger, (NL200, Neuralog System Digitimer LTD), whose threshold was reset for each unit. This allowed for recordings to be made from strands of nerve that contained more than one fibre. This was useful when it was not feasible to further split an already very thin strand of nerve down to a

single fibre. The spike trigger also produced a clear record of the signal from the electrodes by only triggering on action potentials and not on any noise picked up by the electrodes. The output from the spike trigger, as TTL pulses, was then recorded by the magnetic tape recorder. TTL pulses were recorded as an appropriate signal to interface with the computer analysis.

The outputs from the tape of channels 1 to 5, containing flow, volume, carbon dioxide, raw signal from electrodes and TTL of spikes respectively were then put through a 1401 analogue to digital converter, (Cambridge Electronic Design LTD, U.K.). The output from the 1401 was then transferred to an IBM compatible computer, (LC386-25 Akhter UK.) where the data was collected using the Spike2 software package, (Cambridge Electronic Design LTD, UK.).

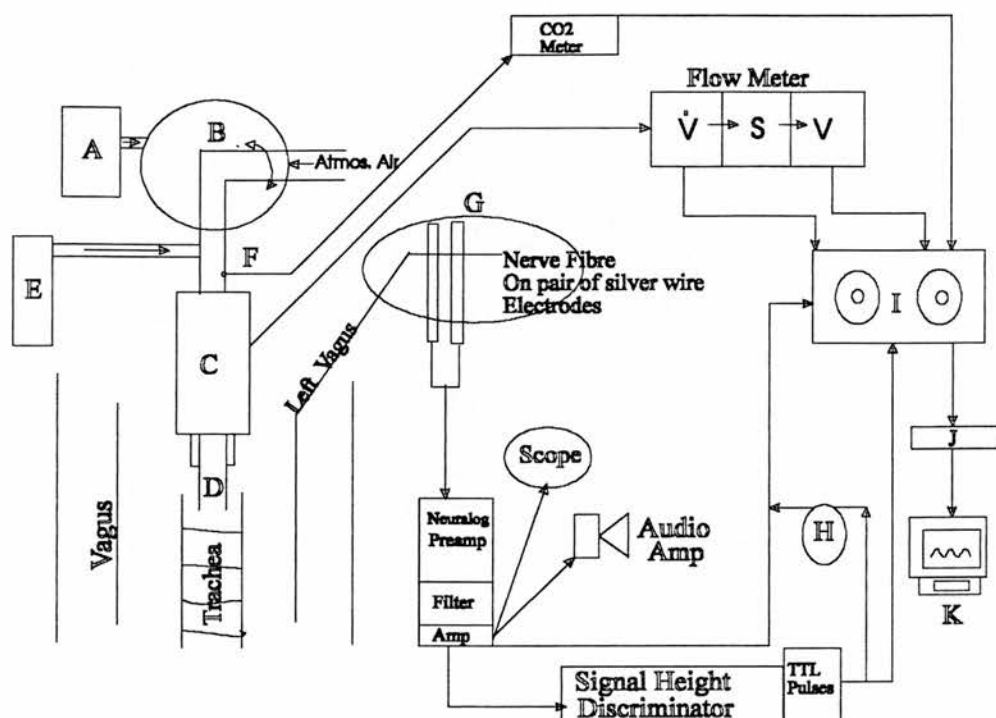


Figure 2-4: Schematic diagram of experimental setup.

Air was held in a 20 litre drum (A) which was held at either positive or negative pressure. A solenoid operated valve (B) allowed atmospheric air or air from the drum to be delivered to rat. The pneumotachograph head (C), connected to the tracheal cannula (D) inserted into the trachea of the rat, measured pressure differences which were transmitted to the flow meter. Flow was electronically integrated to give volume. Flow and volume were recorded on to the 5 channel magnetic tape recorder (I). Carbon dioxide at 4% and 6% was delivered by mixing air with the gas from the carbon dioxide cylinder (E). The left vagus nerve was placed in a tray containing liquid paraffin (G) and single nerve fibres placed across a pair of silver wire electrodes. The activity in the nerve fibre was filtered, amplified and monitored with an oscilloscope and audio amplifier. TTL pulses were produced from the raw activity of the nerve fibre and monitored with oscilloscope (H). These variables were recorded on to magnetic tape. Carbon dioxide was monitored with an infrared CO₂ analyser and also recorded on to (I). Signals from each of the channels of the tape were put through the 1401 analogue to digital converter (J) and then on to the computer (K) where the data was analysed using a script written for the Spike2 data analysis package.

Patterns of discharge under control conditions and in response to sustained lung inflations, deflations and increases of inhaled CO₂ were analysed using a script written for Spike2 (see Appendix 1).

After several fibres had been recorded from each animal, the right vagus nerve was located in the mid-cervical region of the neck. Local anaesthetic (2% Xylocaine) was applied to the nerve on a cotton wool swab and left for 2 minutes to block conduction. The nerve was then cut distal to the block, leaving the animal bilaterally vagotomised. Control breathing in the vagotomised state was recorded 5 minutes after bilateral vagotomy.

At the end of the experiment the rat was killed by an overdose of anaesthetic and the lungs were removed.

SECTION B:- PROTOCOL

EXPERIMENTAL PROCEDURE FOLLOWED IN THE VAGALLY INTACT ANIMAL.

Data analysis included measurements of control breathing in the intact state (when neither vagi were cut) to determine the length of inspiration and expiration, and tidal volume. Five consecutive breaths were analysed. During the two minutes of control breathing that was recorded the number of spontaneously occurring augmented breaths were counted by observing the flow record on the computer. The Hering-Breuer inflation reflex was analysed to measure the length of the apnoea produced by inflation of the lungs, to inflation pressures of 5cm and 10cm of water. The duration of apnoea produced by inflation of the lungs was calculated from the instant of inflation up to the first inspiratory effort, (HB actual value). This value was also expressed with respect to the mean of the two previous respiratory expirations before the inflation was applied, (HB ratio value).

When the lungs were deflated to pressures of 5cm and 10cm of water the deflation reflex was measured in terms of duration of inspiration and expiration of the breaths during the deflation. Breaths 1, 5 and 6 during deflation were analysed. The normalised values of inspiration and expiration for the 1st, 5th and 6th breath occurring during the deflation were calculated. These normalised values were calculated by dividing the inspiratory and expiratory duration of the breaths during deflation by the average time of the inspiratory and expiratory duration of the two breaths that occurred immediately before the deflation was applied, (T_i during deflation was divided by mean T_i before deflation and T_e during deflation was divided by mean T_e before deflation).

Duration of the phases of breathing during 4% and 6% carbon dioxide inhalation were measured as an average of the inspiratory and expiratory durations of the last 5 consecutive breaths that occurred during the two minute period of breathing CO_2 . Tidal volume was also calculated from the analysis of these 5 consecutive breaths.

EXPERIMENTAL PROCEDURE FOLLOWED IN THE UNILATERALLY VAGOTOMISED ANIMAL

In the unilateral vagotomised state, analysis included receptor activity as well as inspiratory and expiratory duration. Breath duration and receptor activity were analysed in the eupnoeic state, the activity of the receptors was also analysed during constant-pressure inflation, and during constant pressure deflation.

For control breathing in the unilaterally vagotomised state five consecutive breaths were analysed. Inspiratory and expiratory duration of these breaths was measured and the activity of the lung receptors during these phases of breathing was analysed. The activity of the lung receptors during a particular breath phase was analysed in terms of:

1. The number of action potentials occurring in each phase
2. The number of action potentials occurring per second, (calculated by dividing the number of A.P.'s per phase by the duration of that phase)
3. The minimum and maximum interval duration between the firing of the action potentials
4. The minimum and maximum frequency of the action potentials
5. The number of action potentials occurring per 100ms bin calculated by dividing the phase of breathing into 100ms bins, (this data was not used for the results).

The activity of the receptors was further analysed by measuring the time at which each action potential fired within a particular phase of breathing, in terms of actual time from the onset of that phase and as percentage time of the total duration of that phase. The actual time from the onset of inspiration or expiration at which each action potential occurred was calculated by the computer using the analysis program. The time at which each of the action potentials occurred was then divided individually by the time taken for that particular breath phase, and multiplied by 100 to give the percentage time of that phase of breathing at which the action potential fired. For example, if the breath phase had a duration of 0.5s and an action potential occurred at 0.25 seconds from the onset of that phase, the percentage time at which that action potential occurred would be $0.25 \text{ divided by } 0.5 = 0.5$ multiplied by 100 = 50%. The percentage times at which the action potential occurred were calculated during the inspiration and expiration periods of five consecutive breaths from each of the receptors analysed. Next the percentage times, of time taken for inspiration, T_i and time taken for expiration, T_e , at which a $1/4$, $1/2$, $3/4$ and all of the action

potentials that fired within that phase had occurred by were found. For example, if a total of 20 action potentials occurred within a particular phase of breathing, the percentage time of that phase of breathing at which 25% of the action potentials had occurred would be when 5 of the 20 action potentials had fired, 50% of them would have occurred when 10 action potentials had been fired, 75% when 15 had fired and 100% when all 20 had fired. The mean value of the average percentage time at which 25, 50, 75 and 100% of the action potentials occurred was then calculated using 5 breaths from each of the receptors.

During inflation, receptor activity was measured for two control breaths before the inflation and then for each of three consecutive 0.25 second periods from when the pressure was applied; and then for the 1st breath after the pressure was removed.

Activity during deflation was measured for two control breaths preceding the pressure and then for the 1st, 5th and 6th breath of the deflation and finally for two breaths immediately after the pressure was removed.

Activity during administration of carbon dioxide was analysed for five consecutive breaths at the 4% and 6% level.

EXPERIMENTAL PROCEDURE FOLLOWED IN THE BILATERALLY VAGOTOMISED ANIMAL.

The inspiratory and expiratory duration of 5 consecutive breaths were analysed. Statistical tests were performed on the data and presented as the mean value plus and minus standard error of the mean.

The computer script written for the Spike2 analysis package, measured the duration of T_i and T_e by measuring the time between two cursors, see Figure 2-5. The cursors were positioned manually. For measuring the time of inspiration, (T_i) the first cursor was positioned at the onset of inspiration and the second cursor at the end of inspiration; similarly for measuring the duration of time taken for expiration, (T_e) the first and second cursors were placed at the beginning and end of expiration respectively, (see Figure 2-5).

The area under the curve made by the flow trace was integrated by the computer to give a value for volume.

Between the two cursors, in the unilaterally vagotomised state, the number of action potentials were counted by the computer and their instantaneous frequencies calculated from their inter-spike interval times. The position of the action potentials from the onset of the phase of breathing i.e. the first cursor position, were also calculated by the computer.

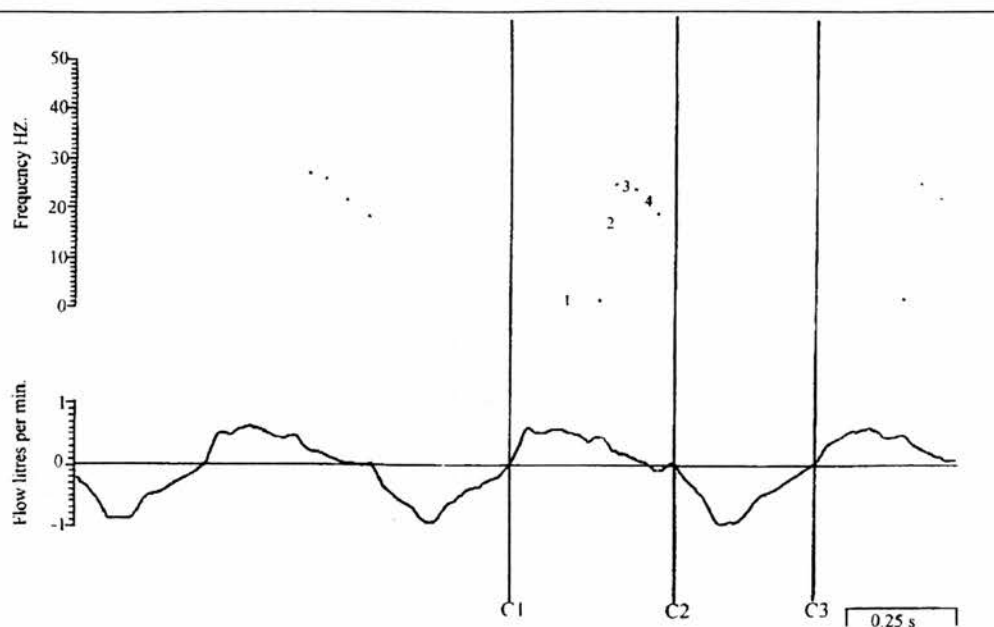


Figure 2-5: Data as displayed on the computer screen during analysis with the script written for the Spike2 analysis package.

Initially the first cursor is set at position C1, the beginning of inspiration and the second cursor is set at C2, the end of inspiration, ($C2 \text{ position} - C1 \text{ position} = T_i$). To measure the duration of expiration the cursors were then reset at positions C2 and C3 ($C3 \text{ position} - C2 \text{ position} = T_e$). The dots on the diagram mark the positions at which the action potentials occurred and the numbers (1-4) represent the intervals between the action potentials. (1) is the time from the beginning of the breath phase to the occurrence of the first action potential, shown by the first dot, (2) is the spike interval time between the first and second action potential occurring in that phase, (3) is the spike interval time between second and third action potential and (4) is the spike interval between the third and fourth action potentials.

The instantaneous frequency of each action potential between the cursors was calculated using the formula $1/\text{action potential interval time}$. For example the instantaneous frequency of the third action potential occurring between C1 and C2 was calculated as $1/\text{action potential interval (2)}$.

The position at which each of the action potentials occurred from the onset of each phase C1 in T_i and C2 in T_e was also calculated by the computer. Interval (1) was only used for positional purposes to find where the first action potential occurred in each phase, and the instantaneous frequency was ignored as this was not a true action potential interval time, but merely the interval from position C1 to the first action potential.

CALIBRATIONS.

The pneumotachograph was calibrated at the beginning of each experiment. The apparatus was calibrated for flow, volume and carbon dioxide. Air at flow rates of 1 and 2 litres per minute was passed through the pneumotachograph and the volt equivalent deflections produced by these flow rates were recorded on the magnetic tape, transferred on to the computer and analysed. The flow rates were checked to ensure that linear increases in flow produced linear increases in the volt record. The known flow rates could then be used to calculate the unknown flow rates at which the rats breathed.

A series of known volumes (1-10ml) of air were forced through the pneumotachograph and the volt deflections they produced were recorded on tape and transferred to the computer. The deflections produced by these known volumes were then used to find out the tidal volume of the rats.

VERIFICATION OF ACTION POTENTIAL FREQUENCY CALCULATED BY THE SPIKE2 ANALYSIS PROGRAM.

Waves of known frequency 0, 1, 10, 100, 500 and 1000 HZ were produced by a signal generator. Sigmoidal waves formed on the signal generator were put through the Neuralog spike trigger and oscilloscope. The output from the spike trigger was then put through the 1401 analogue to digital converter and transferred to the computer using the Spike2 data capture program. The data was displayed using the Spike2 analysis program. The number of pulses collected by the computer over a known period was manually counted to verify that the computer had collected the signals produced by the signal generator accurately. The spikes displayed on the computer were counted over 10 seconds for the 1 Hz wave, 1 second for the 10 and 100 Hz waves and 0.1 seconds for the 500 and 1000 Hz waves. The computer accurately recorded the correct number of spikes.

The spikes were then counted using the Spike2 analysis program to verify that it accurately calculated spike frequencies.

STATISTICAL METHODS

The aim of the thesis was to find out if there were differences in pattern of breathing and vagal lung receptor activity between two groups of rats. One group was a control group while the other group of rats had been induced with emphysema. To answer this question it was necessary, after collection of the data, to analyse the results statistically to find if any meaningful differences between the two groups existed.

The measurements made of pattern of breathing, reflex responses to lung inflation and deflation, response to inhalation of CO₂ and vagal lung receptor activity were compared between the two groups of rats. The two groups were sex, age and weight matched. The numbers of rats in each sample were adequate to test the differences produced (15 in the controls and 18 in the emphysematous), although the total number of vagal lung receptors recorded from each sample was larger, 85 in the controls and 99 in the emphysematous.

Statistical tests were necessary to test the probability that the observed differences were due chance. Statistical tests were carried out to compare data recorded from the control and emphysematous groups to find if there was any significant differences between the mean values in the two samples.

To make a valid comparison between the data in the two samples, unpaired Student's t-tests were employed as they provide a simple yet rigorous test of significance between the means. More complex statistical tests were not thought particularly useful as data that fails to yield a significant result when subjected to simple tests but do so after a refined and complex analysis is often suspect, (Swinscow, 1990). Therefore to elucidate differences that have practical importance the simple statistical methods are often more appropriate. Statistical advice was obtained from Dr. H. Brown of the University of Edinburgh's Statistics Department, whose contribution is gratefully acknowledged.

Mean values and the standard error of the mean for each set of data from the control and emphysematous groups were calculated. The standard errors of the means were then used to study the significance of the difference between the two means using Student's t-test, the preferred statistical test to compare means when the number of observations is small (lower than 60) as more chance variation is allowed for in this test, (Swinscow, 1990). The null hypothesis that there was no difference between the mean values in the results from the control rats and those from the emphysematous rats was tested using the standard equation, see (Swinscow, 1991).

The number of degrees of freedom was calculated as $(n_1-1) + (n_2-1)$ and the value of t entered in a table of the t distribution. The degree of probability that there is no difference between the means of the control and emphysematous data was found from the table. Probabilities that reached the conventional 5% level or less indicated the null hypothesis, that there was no significant difference between the means, was somewhat unlikely.

Data of the number of different types of receptors was statistically analysed. This data was analysed to find if there was a statistical significant difference in the number of the various types of receptors recorded from in the control and emphysematous rats. The Chi-squared test was used to test the null hypothesis that there was no association between the two sets of data.

Statistical analysis was performed on the data to test the relationship between neuro-physiological changes and the severity of the disease. These tests were necessary to assess if the extent of the disease in terms of lung compliance had any relationship to the either the strength of the Hering-Breuer reflex or the activity of slowly adapting receptors in eupnoea and during lung inflation. The statistical analysis used to test the relationship was the non-parametric Spearman's rank correlation where the resulting statistic has a distribution which is not dependant on the distribution of the original variables, (Bland, 1996). Since there was only four sets of data available for this statistical analysis these could not have been assumed to have a normal distribution. The null hypothesis that there was no relationship between the extent of the disease and SAR activity was therefore tested using the Spearman's rank correlation. Spearman's statistic ρ was calculated as follows:- ranks for the two variables were found and then the formula for the product moment correlation was then applied to these ranks. For the equation used to calculate ρ , see Bland, 1996. However with statistical advice it was decided that with such a low value of n for the set of data it was not possible to reliably test the significance of the Spearman's rank correlation coefficient.

CHAPTER 3

GENERAL RESULTS.

The results are presented in separate chapters which are described below. The unpaired Student's t-test was used to determine significance of difference between means of sample groups. A value of $P < 0.05$ was considered to be a significant difference. Except where specifically mentioned means are quoted \pm the standard error of the mean.

INDUCTION OF EMPHYSEMA IN RATS.

RESULTS OF INSUFFLATION.

The endotracheal administration of Papain induced emphysematous-like lesions. Since this method of induction was successful no attempt was made to induce emphysema by aerosol administration of Papain. Performing the preliminary investigations into suitable doses of Papain, a dose of 12mg/100g of body weight was decided upon as this produced significantly different alveolar mean linear intercept measurements compared to the controls, (see mean linear intercept results), without causing deaths. The Papain was administered in 0.05ml of physiological saline per 100g body weight. This was the minimum amount of fluid that the Papain could be suspended in and still be fluid enough to flow. When the Papain was suspended in a larger volume of solution respiratory distress was noted and some rats died.

RESULTS OF PRELIMINARY AEROSOL TESTS.

A method of delivering aerosols of Papain was developed in case the emphysema produced by insufflation proved unsatisfactory. The ultrasonic nebuliser produced from a modified Pifco domestic humidifier successfully aerosolised solutions. Although it did not prove necessary to use, the results were of interest and the instrument sufficiently novel to warrant publication, (Pirie, 1993).

The amount of aerosol produced, in terms of volume of air and droplet density, could be varied by altering the power supplied to the air fan and ultrasonic transducer respectively using the original controls of the humidifier.

The focused energy of the transducer was sufficiently great, at full power setting, to aerosolise 4ml of solution per minute. The characteristics of a room temperature distilled water aerosol were:-

90% of droplets below 6 microns in diameter

50% of droplets below 5 microns

10% of droplets below 4 microns.

These characteristics are shown in Figure 3-1.

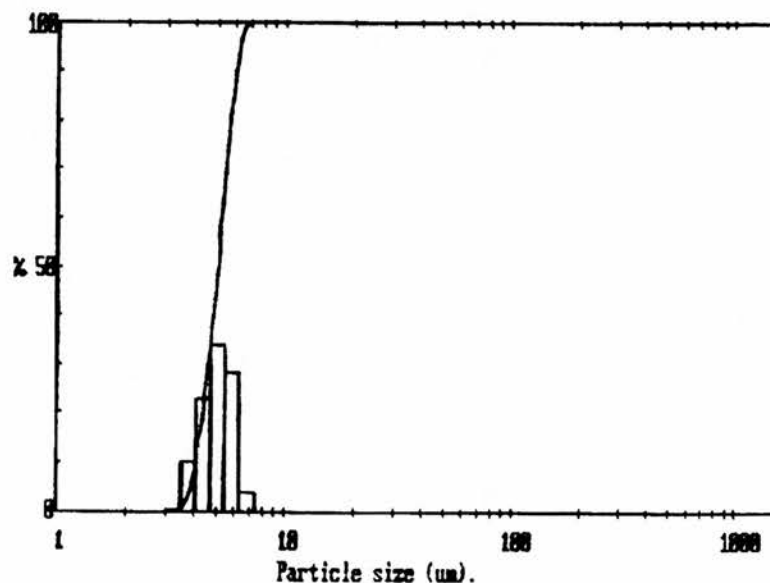


Figure 3-1: *Size profile of aerosolised saline particles produced from the nebuliser at room temperature.*

The data was generated as particle mass histograms comprising 10 size bands over the range 7.30-1.93 microns. The physical basis of this technique and its use for evaluating particle size distributions from nebulisers has been described elsewhere, (Hardy, Newman and Knoch 1993).

Size of Droplets in microns	% under	% in band
7.30	100	0.00
6.30	96.0	28.3
5.43	67.7	34.0
4.68	33.8	23.1
4.05	10.6	10.30
3.48	0.36	0.36
3.02	0.00	0.00
2.60	0.00	0.00
2.23	0.00	0.00
1.93	0.00	0.00

Table 3-1: Droplet size of particles, in microns, generated from nebuliser showing the percentage number of particles of that size.

No droplets larger than 7.30 microns were detected and none smaller than 3.02 microns.

Of the total number of particles aerosolised 90% of them had a particle size of 6.02 microns or less, 50% of them had a particle size of 5.06 microns or less, and 10% had a particle size of 4.03 or less.

The size distribution was not affected by the power setting of the transducer and compares favourably with commercial ultrasonic nebulisers in which median droplet diameters ranged from 5.2-6.9 microns, (Lourenco and Cotromanes, 1982).

Maximum alveolar and bronchial deposition is obtained with droplet sizes in the range of 2-4 and 5-10 microns respectively in humans, (Sterk, Plomp, Van de Vate and Quanjer, 1984). Our inexpensive nebuliser produces particles where 50% are less than 5 microns in diameter and 90% are less than 6 microns. The optimum size range for diffuse pulmonary deposition is about 2-6 microns depending on breathing pattern and airway geometry, (Sterk, Plomp, Van de Vate and Quanjer, 1984).

RESULTS OF ELASTOLYTIC ASSAY.

100mg/ml of a solution containing Congo red-elastin and Papain gave an absorbance of 0.064 after 20 minutes. This was the time at which 50% digestion of the elastin had occurred, measured as a release of 50% of the Congo red into the solution. From the standard curve obtained from the known concentrations of pure elastase, an elastase equivalent of 0.036mg/ml was required to give an absorbance reading of 0.064. The elastase was much more potent than the crude Papain at digesting elastin. The elastolytic activity of the elastase was 2777 times greater than the crude Papain.

ASSESSMENT OF EMPHYSEMA.

MEAN LINEAR INTERCEPT METHOD.

The sections taken from the lungs of the treated rats, when viewed under the microscope, appeared to be clearly different from the sections taken from the lungs of the control rats. The treated rats had larger airspaces and more broken alveoli walls than the control rats, (see photomicrographs of control compared to diseased lung).

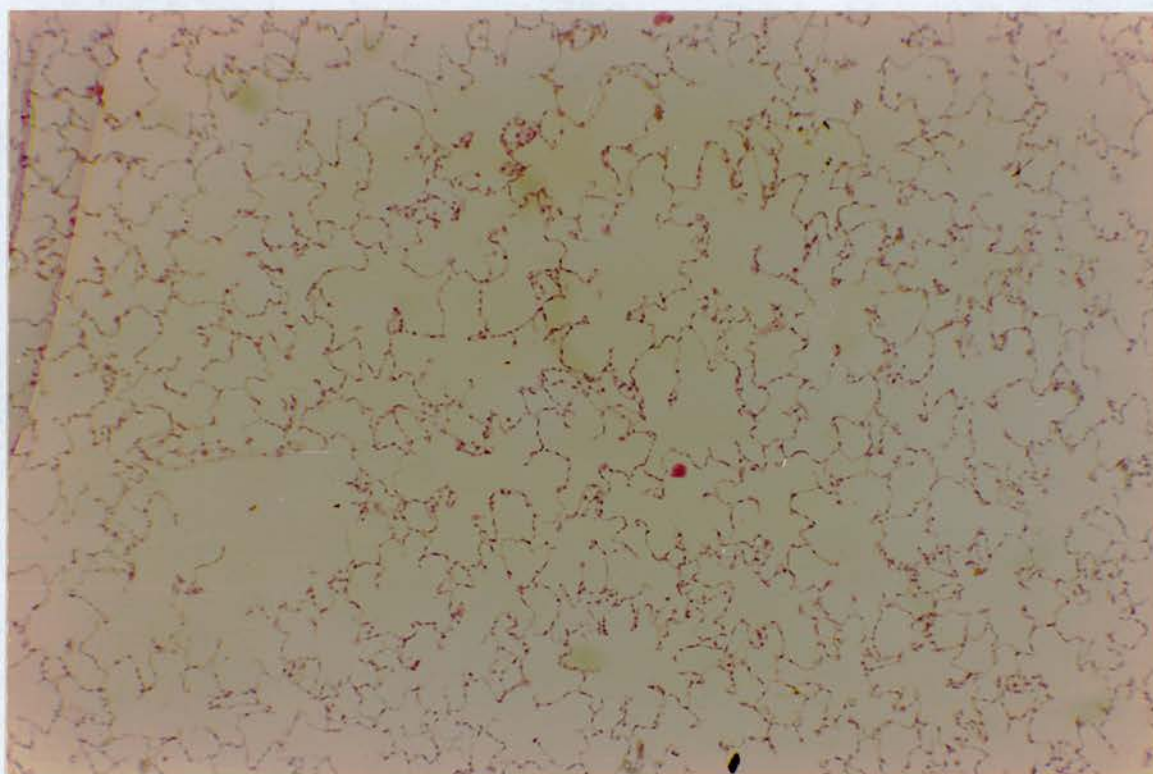


Figure 3-2: *Photomicrograph of alveoli from control rats. Original mag. x 60.*

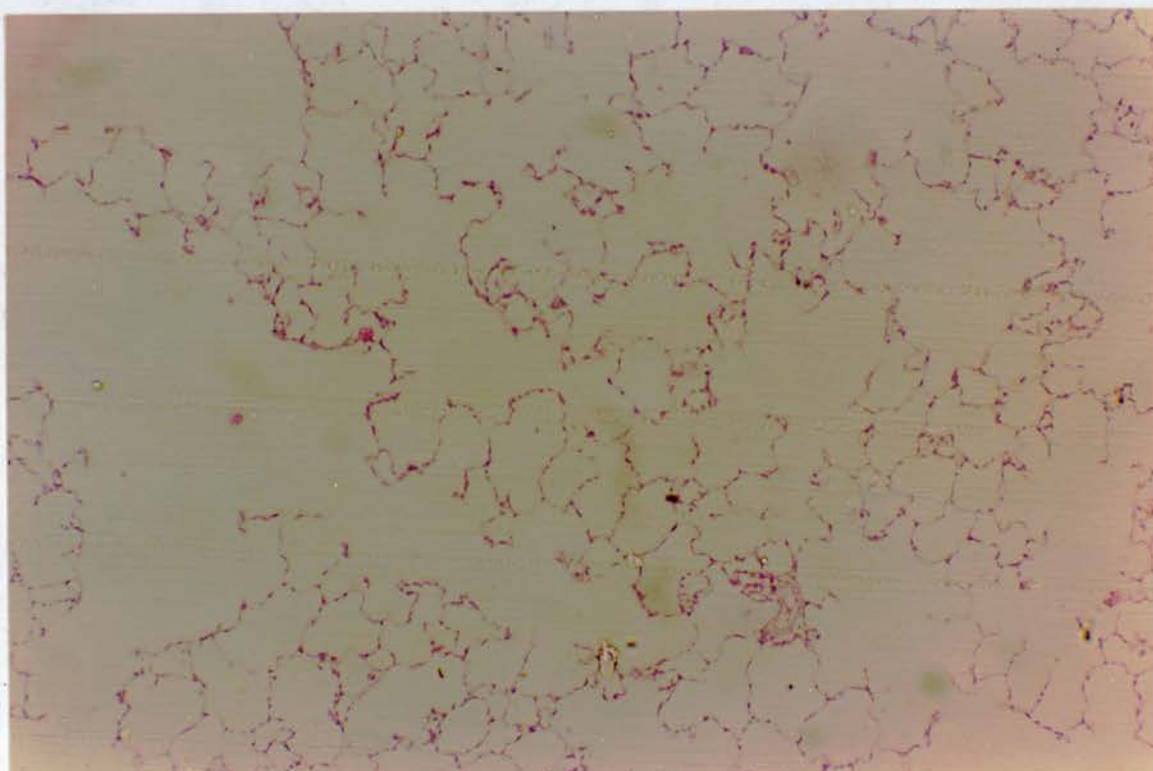


Figure 3-3: *Photomicrograph of alveoli from Papain treated rats. Original mag. x 60.*

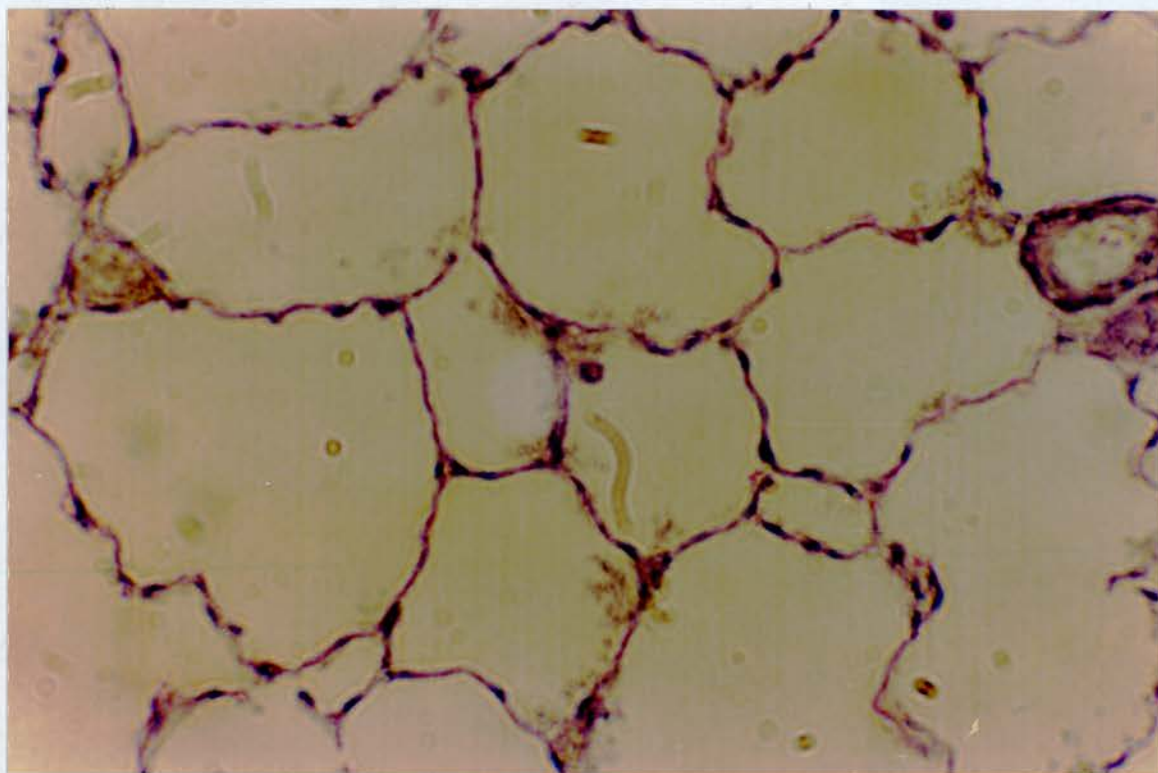


Figure 3-4: *Photomicrograph of alveoli from control rats.* Original mag. x 300.

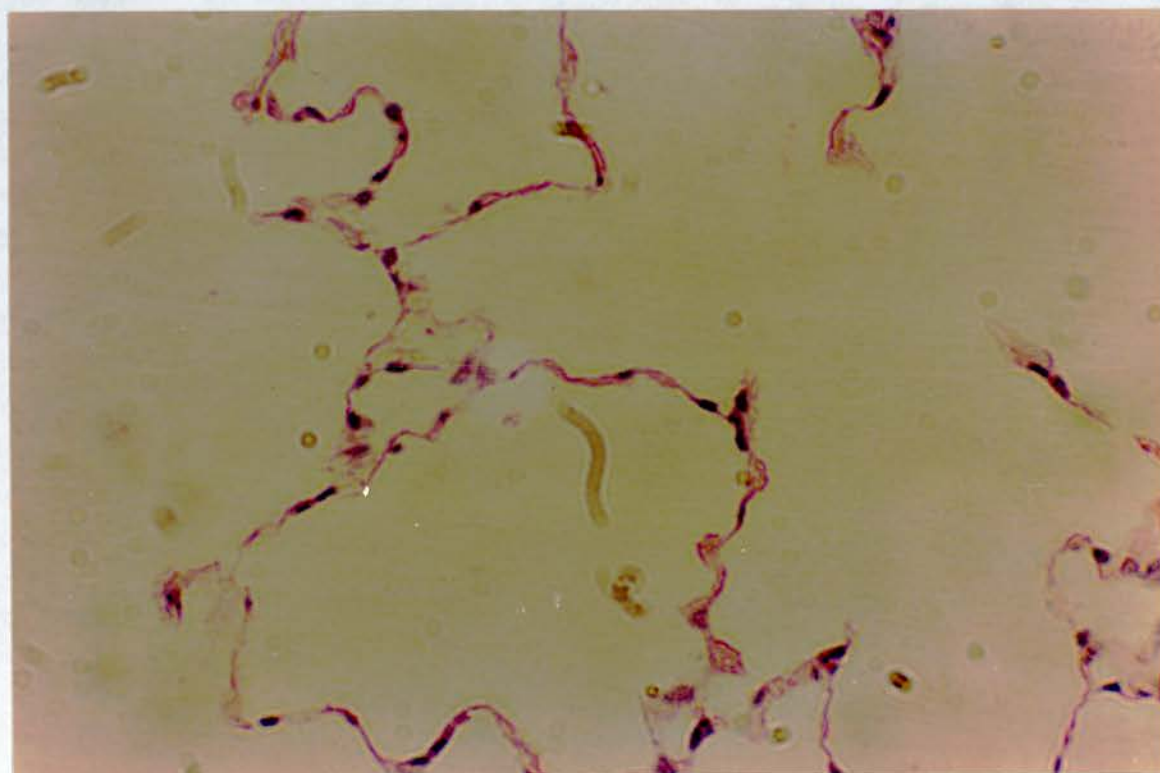


Figure 3-5: *Photomicrograph of alveoli from Papain treated rats.*
Original mag. x 300

The mean linear intercept, (L_m) value for the control rats was $81.5 \pm 3.08 \mu\text{m}$ ($n=7$) and $109.3 \pm 2.70 \mu\text{m}$ ($n=31$) for the Papain treated rats. The results show a statistically significant difference between the mean linear intercept for the control and Papain treated rats at $P<0.01$.

RESULTS OF THE BLOOD GAS ANALYSIS.

There was no statistically significant difference in the partial pressure of oxygen in the arterial blood sampled from the control and the emphysematous rats. The control rats had a PO_2 of $13.4 \pm 0.9 \text{ kPa}$, ($n=5$) and the diseased rats had a slightly lower PO_2 of $12.4 \pm 0.5 \text{ kPa}$, ($n=15$).

The PCO_2 of arterial blood taken from the control rats was not statistically significantly different to the PCO_2 of the arterial blood of the emphysematous rats. The control rats had a PCO_2 of $5.83 \pm 0.3 \text{ kPa}$, ($n=5$) and the emphysematous rats had a slightly higher PCO_2 of $6.46 \pm 0.3 \text{ kPa}$, ($n=15$).

RESULTS OF COMPLIANCE MEASUREMENTS.

STATIC COMPLIANCE WITH THE LUNGS IN SITU.

When the volume / pressure curves were plotted for both groups of rats the emphysematous rats had steeper curves than the curves produced by the control rats, see Figures 3-6 and 3-7.

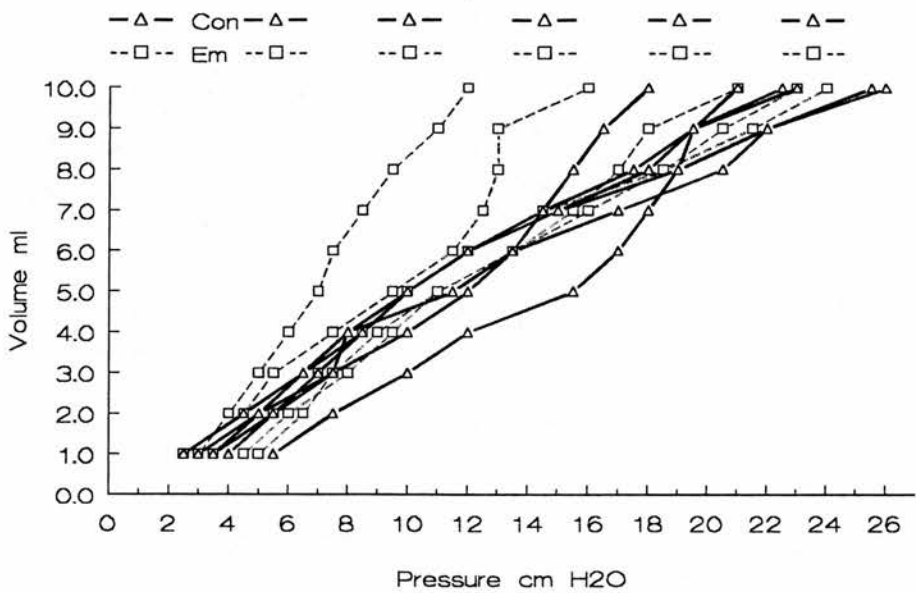


Figure 3-6: *Volume / pressure inflation curves from six control and six emphysematous rats. The slope of the individual curves gave the static compliance value of the lungs from an individual rat.*

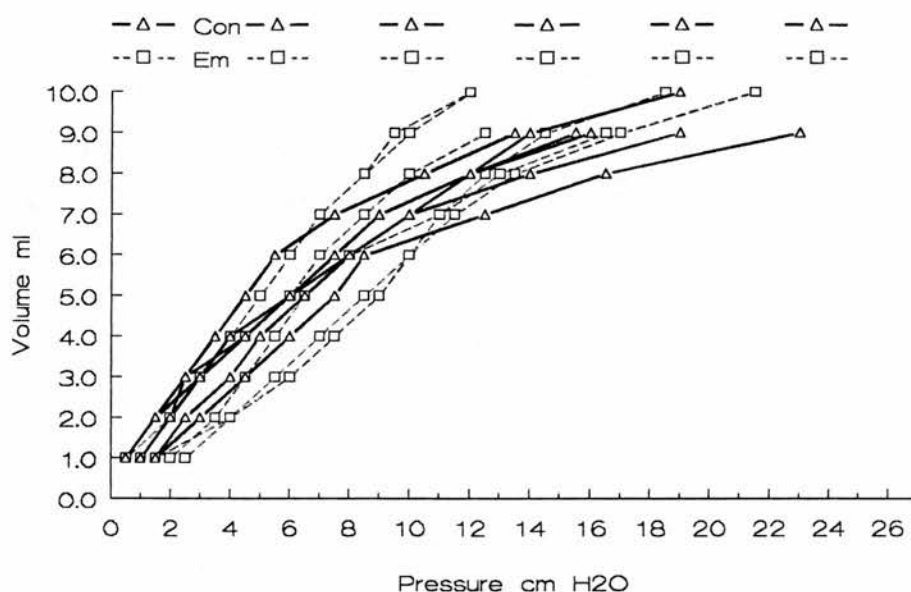


Figure 3-7: *Volume / pressure deflation curves from six control and six emphysematous rats. The slope of the individual curves gave the static compliance value of the lungs from an individual rat.*

The slope of each individual curve was calculated. The mean value of the slope of the curves for each of the two groups was calculated from these individual slope values. The mean static compliance of the control lungs in situ, measured from the slope of the volume/pressure inflation curve, was 0.41 ± 0.04 ml/cmH₂O, (n=6) and 0.66 ± 0.08 ml/cmH₂O, (n=6) in the emphysematous rats. This represented a statistically significant difference at $P < 0.02$. The mean static compliance of the control lungs in situ measured from the slope of the volume/pressure deflation curve was 0.61 ± 0.02 ml/cmH₂O, (n=6) and 0.82 ± 0.03 ml/cmH₂O, (n=6) in the emphysematous rats. This represented a statistically significant difference at $P < 0.01$.

PATTERN OF BREATHING IN INTACT STATE.

Anaesthetised breathing rate before insertion of the tracheal cannula was measured by counting the number of breaths taken by the animal in one minute. This was repeated several times for each of the animals used. Twelve of the control rats had their breathing rate estimated in this manner. The mean frequency was found to be 96.1 ± 3.5 breaths per minute. The breathing frequency of nine treated rats, four weeks after Papain administration, was similarly estimated. Before tracheal cannulation the mean breathing frequency of these treated rats was found to be 99.2 ± 1.43 breaths/minute. The difference in breathing frequency between control and diseased groups, before tracheal cannulation, was not significantly different when tested for statistical significance using the Student's t-test.

On tracheal cannulation the breathing frequency fell in both groups to 81.4 ± 6.5 breaths/minute, ($n=7$) in the controls and 90.57 ± 4.21 breaths/minute, ($n=7$) in the diseased. This difference in breathing frequency between control and diseased groups was not statistically significantly different.

With the pneumotachograph connected to the tracheal cannula, the frequency of breathing was increased to a level of 105.9 ± 5.2 breaths/minute, ($n=7$) in the untreated rats and 112.0 ± 4.9 breaths/minute, ($n=7$) in the diseased. These values, close to the frequencies in the intact anaesthetised states, were not significantly different from each other.

From the flow records, the duration of the breathing phases of the tracheostomised rats were measured by computer analysis using the script written for the Spike2 software package. Sixteen control rats were found to have a mean breathing cycle time of 0.49 ± 0.02 s. Their mean inspiratory time was 0.24 ± 0.01 s, ($n=16$) while mean expiration time was 0.25 ± 0.01 s, ($n=16$). Mean breathing cycle time of 18 emphysematous rats was 0.54 ± 0.03 s. Mean inspiratory time for the emphysematous rats was 0.25 ± 0.01 s, ($n=18$). Mean expiratory time was 0.29 ± 0.02 s, ($n=18$). There was no statistically significant difference between the means of the total breath time, or inspiration and expiration time between the control and the treated rats, (see Figure 3-8).

	Controls	Emphysematous
Ti in seconds	0.24 +/- 0.01	0.25 +/- 0.01
Te in seconds	0.25 +/- 0.01	0.29 +/- 0.02
Tt in seconds	0.49 +/- 0.02	0.54 +/- 0.03
V _T in ml	2.83 +/- 0.20	2.85 +/- 0.16

Table 3-2: Pattern of breathing in control and emphysematous rats during eupnoea.

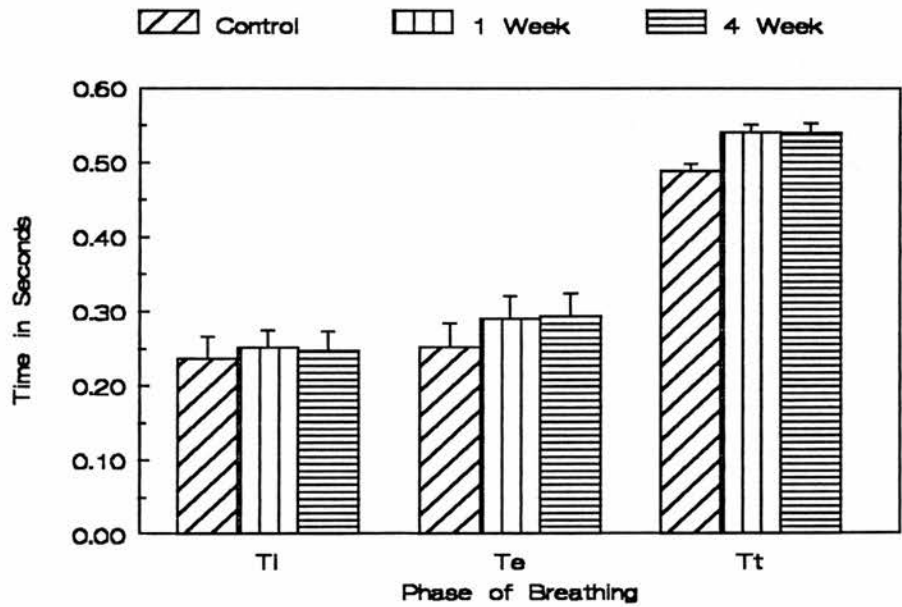


Figure 3- 8: Eupnoeic breathing in control and emphysematous rats in the intact state. Duration of inspiration (Ti), expiration (Te) and the total breath (Tt).

TIDAL VOLUME IN EUPNOEA WITH VAGI INTACT.

Expiratory tidal volume was measured in the control and diseased rats during eupnoea. Under these conditions there was no difference between the mean tidal volume calculated for each group. The controls had a tidal volume of 2.83 ± 0.20 ml and the emphysematous rats had a tidal volume of 2.85 ± 0.16 ml.

VARIABILITY IN BREATHING PATTERN.

To assess the variability in breathing pattern of the two groups of rats the number of augmented breaths spontaneously occurring in the animals was counted. An illustration of an augmented breath taken from the flow record is shown in Figure 3-5. From this illustration it can be seen that an augmented breath was easily distinguishable from normal eupnoeic breaths by its augmented inspiration and prolonged expiration. The breathing following an augmented breath was rapid and shallow, (in Figure 3-9, compare the 4 breaths shown before the augmented breath to the 5 occurring after this breath). Although the illustration is of an augmented breath from a control rat it provides a typical example of an augmented breath from both the groups of rats.

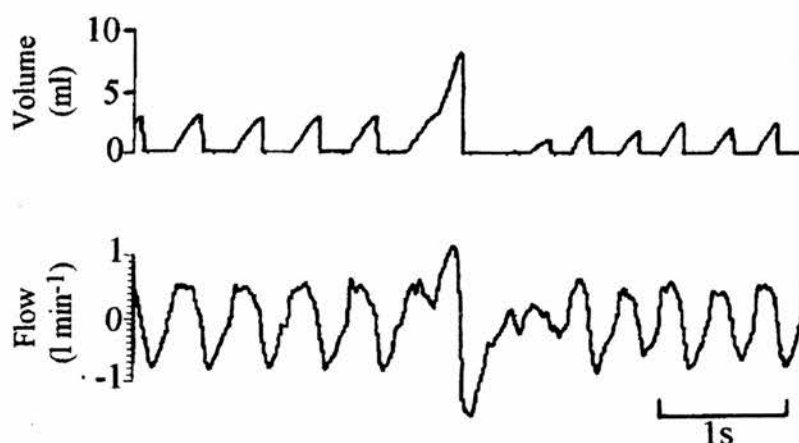


Figure 3-9 : *Typical augmented breath from a control rat.*

The number of augmented breaths occurring, in two minute periods of eupnoeic breathing recorded from 14 control and 17 emphysematous rats, were counted manually from the flow record displayed on the computer. The 14 control rats took a total of 3 augmented breaths during the 28 minutes of breathing analysed. The 17 emphysematous rats took a total of 2 augmented breaths in the 34 minutes of breathing analysed. Either a rat took no augmented breaths or it took only one such breath during a two minute period. The diseased rats on average took a lower number of augmented breaths during eupnoeic breathing.

PATTERN OF BREATHING WHEN THE VAGI WERE CUT.

In the unilaterally vagotomised state breathing pattern was recorded and analysed, together with receptor activity from single fibres of the vagus nerve. The pattern of breathing was also recorded and analysed when the rats were bilaterally vagotomised. The breathing during vagotomy was analysed in 10 control rats and in 18 emphysematous rats.

When the rats were unilaterally vagotomised, breathing frequency fell as the duration of both T_i and T_e increased in both groups of rats. However the unilaterally vagotomised emphysematous rats had a slightly higher frequency of breathing than the control unilaterally vagotomised rats, as cutting one of the vagi had a slightly less marked effect on increasing the duration of T_i and T_e . In the emphysematous rats, breathing slowed less after unilateral vagotomy than it did in the control rats, due to both a shorter T_i and shorter T_e of the diseased rats compared to the controls, see Figure 3-10. Bilaterally vagotomised emphysematous rats again had a higher breathing frequency than the controls; this was due to a shorter T_e in the emphysematous rats. The results of the pattern of breathing in the eupnoeic, unilateral and bilateral vagotomised state are shown in Figure 3-10.

	<u>Control Rats</u>		<u>Emphysematous Rats</u>	
	Unilateral	Bilateral	Unilateral	Bilateral
Ti in seconds	0.38+/-0.08	0.61+/-0.01	0.36+/-0.07	0.63+/-0.07
Te in seconds	0.47+/-0.01	1.42+/-0.20	0.45+/-0.01	1.30+/-0.14
	n=135 breaths	n=50 breaths	n=200 breaths	n=90 breaths

Table 3-3: *Pattern of breathing of control and emphysematous rats in the unilateral and bilateral vagotomised state.*

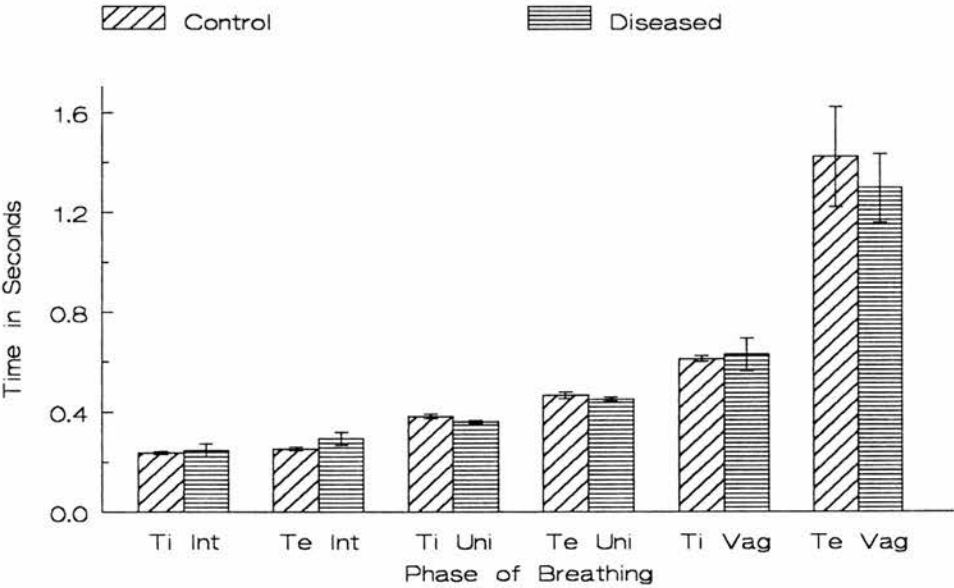


Figure 3-10: *Intact, unilateral and bilateral vagotomised pattern of breathing in control and 4 week emphysematous rats, shown in terms of duration of inspiration, (Ti) and duration of expiration, (Te).*

REFLEXES.

RESPONSE TO INFLATION PRESSURE.

When their lungs were inflated to positive pressures of 5 and 10cm of water the rats from both groups stopped breathing, showing a classical Hering-Breuer inflation reflex. In control rats the apnoea produced by a positive pressure of 5cm of water was $2.68 \pm 0.48s$, ($n=27$). In the emphysematous rats an apnoea of $6.34 \pm 1.4s$, ($n=36$) was produced. The apnoea evoked by the Hering-Breuer inflation reflex showed a statistically significant difference between the mean values for the control and treated rats at $P<0.05$.

The duration of the Hering-Breuer pause was divided by the expiratory time of the immediately preceding breath before inflation. This value, which was called the Hering Breuer ratio, was calculated to take into account the differences in eupnoeic expiratory time of the two groups of animals. For each rat the lungs were inflated twice to each of the pressures. The mean value calculated from the individual Hering-Breuer ratios of each rat gave a value of 9.18 ± 1.8 , ($n=27$) for the control rats and 19.67 ± 3.1 , ($n=36$) for the emphysematous rats, at an inflation pressure of 5cm of water. This was found to represent a statistically significant difference between the mean values of the Hering-Breuer ratios at $P<0.01$. The response to an inflation pressure of 5cm of water was 2.4 times greater in the diseased rats than in the controls, (see Figure 3-11).

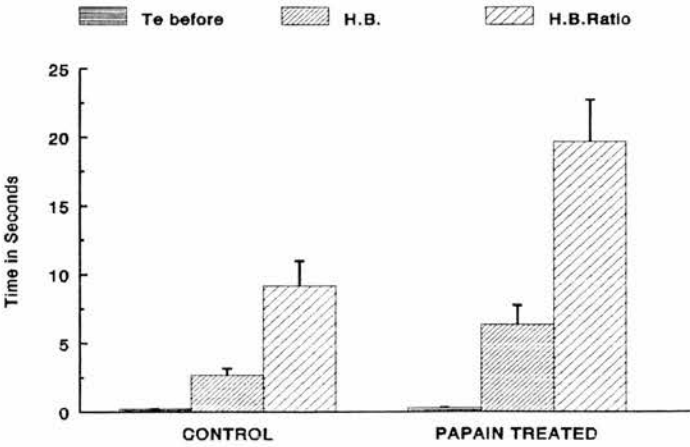


Figure 3-11: *Response of control and emphysematous rats to an inflation pressure of 5cm of water. Te before is the expiratory duration of the breath immediately before inflation, H.B. is the absolute Hering Breuer pause and H.B. ratio is H.B./Te before.*

The mean absolute Hering-Breuer pause for the control rats when their lungs were inflated to positive pressures of 10cm of water was 17.3 +/- 1.9s, (n=27) and for the diseased rats 27.80 +/- 2.8s, (n=36). This was a statistically significant difference at $P<0.01$. The results of the Student's t-test between the means of the Hering-Breuer ratios produced by positive inflation pressures of 10cm of water also showed statistically significant differences at $P<0.05$. The results gave a mean Hering-Breuer inflation ratio of 58.37 +/- 6.8, (n=27) for the controls and a much greater pause ratio of 96.19 +/- 8.3, (n=36) in the emphysematous rats, (see Figure 3-12). The response to an inflation pressure of 10cm of water was 1.6 times greater in the emphysematous rats than in the controls.

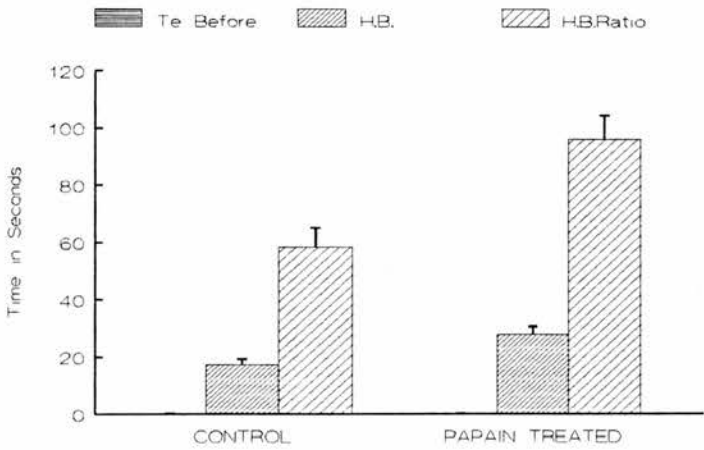


Figure 3-12: Response of control and emphysematous rats to an inflation pressure of 10cm of water. "Te before" is the expiratory duration of the breath immediately before inflation, H.B. is the absolute Hering Breuer pause and H.B. ratio is H.B./Te before.

RESULTS OF RESPONSE TO DEFLATION PRESSURE.

Negative intratracheal pressure of 5 or 10cm of water altered the pattern of breathing. Inspiratory time increased in both the control and the diseased rats and expiratory time decreased in both the groups. The 1st, 5th and 6th breath after the deflation were analysed in terms of T_i and T_e . The results are shown in Tables 3-2 & 3-3 for negative 5 and 10cm deflation respectively and in Figures 3-13 to 3-16.

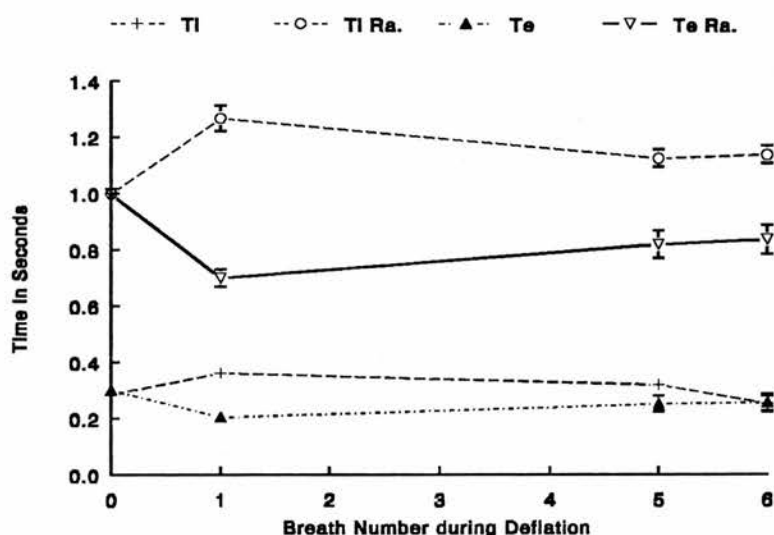


Figure 3-13: Response to a deflation pressure of 5cm of water in the control rats. T_i is the inspiratory duration, T_e is expiratory duration, $T_i Ra$ is T_i during deflation divided by eupnoeic T_i immediately before deflation and $T_e Ra$ is T_e during deflation divided by eupnoeic T_e immediately before deflation.

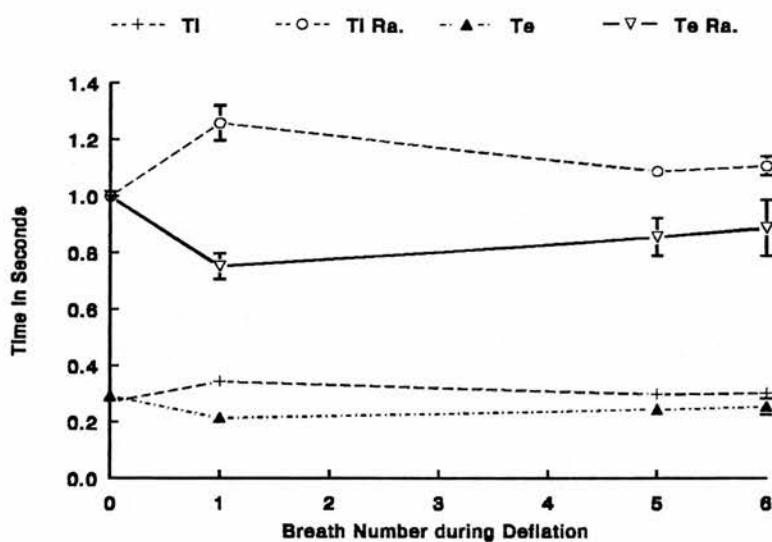


Figure 3-14: Response to a deflation pressure of 5cm of water in the emphysematous rats.

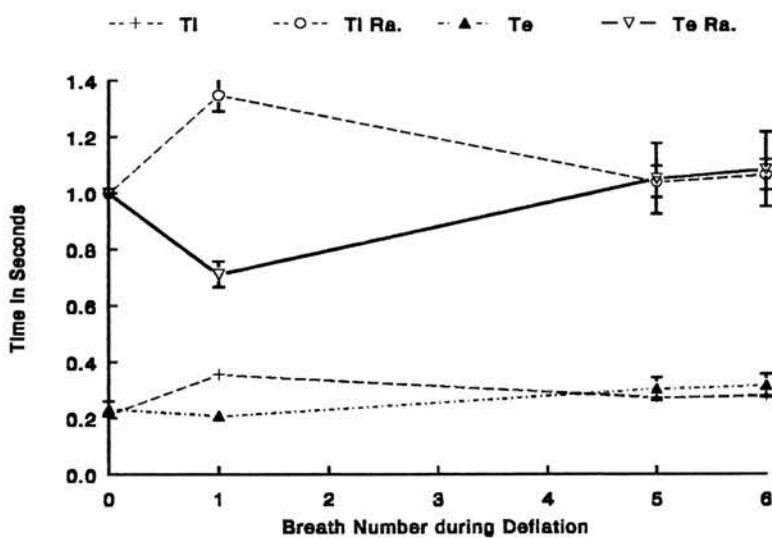


Figure 3-15: Response to a deflation pressure of 10cm of water in the control rats.

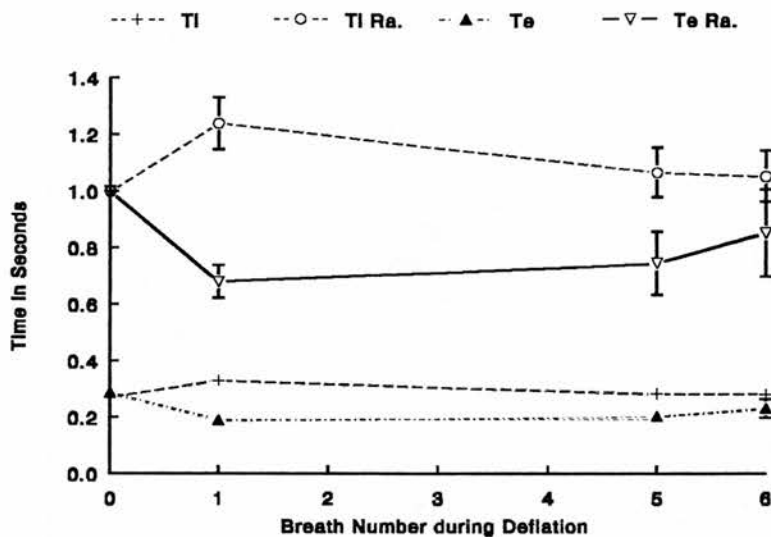


Figure 3-16: Response to a deflation pressure of 10cm of water in the emphysematous rats.

Breath Number	Control rats n=28 Deflation tests		Emphysematous rats n=28 Deflation tests	
	Ti in seconds	Te in seconds	Ti in seconds	Te in seconds
Eupnoeic Breath	0.285 +/- 0.01	0.296 +/- 0.01	0.273 +/- 0.01	0.298 +/- 0.01
1st of Deflation	0.355 +/- 0.02	0.212 +/- 0.01	0.333 +/- 0.01	0.214 +/- 0.01
5th of Deflation	0.321 +/- 0.01	0.254 +/- 0.03	0.299 +/- 0.01	0.252 +/- 0.02
6th of Deflation	0.325 +/- 0.01	0.262 +/- 0.03	0.293 +/- 0.01	0.256 +/- 0.02

Table 3-2: Time of breath phases, (Ti and Te) immediately before and during deflation to 5cm of water pressure in control and emphysematous rats.

Breath Number	Control rats n=28 Deflation tests		Emphysematous rats n=28 Deflation tests	
	Ti in seconds	Te in seconds	Ti in seconds	Te in seconds
Eupnoeic Breath	0.275 +/- 0.01	0.294 +/- 0.01	0.271 +/- 0.01	0.290 +/- 0.01
1st of Deflation	0.331 +/- 0.02	0.204 +/- 0.02	0.324 +/- 0.02	0.293 +/- 0.05
5th of Deflation	0.279 +/- 0.01	0.288 +/- 0.05	0.262 +/- 0.02	0.279 +/- 0.03
6th of Deflation	0.274 +/- 0.01	0.264 +/- 0.04	0.263 +/- 0.02	0.290 +/- 0.03

Table 3-3: *Time of breath phases (Ti and Te) immediately before and during deflation to - 10cm of water pressure in control and emphysematous rats.*

The data for the duration of inspiration and expiration during deflation were normalised by dividing the Ti and Te during deflation by the immediately preceding eupnoeic Ti or Te respectively. This was to avoid differences in the duration of the breath phases during eupnoeic breathing influencing any changes in Ti and Te due to deflation. A normalised value of 1 would indicate that no change in the phase of breathing had occurred compared to the immediately preceding eupnoeic phase, a value greater than 1 would be obtained if the phase increased in duration and a value below 1 would be obtained if the duration of the phase was decreased. The results are shown in Tables 3-4 & 3-5 for 5 and 10cm deflation pressures respectively.

	Control rats n=28 Deflation tests		Emphysematous rats n=28 Deflation tests	
Normalised Value	Ti Deflation / Eupnoeic Ti	Te Deflation / Eupnoeic Te	Ti Deflation / Eupnoeic Ti	Te Deflation / Eupnoeic Te
Breath Number				
1st of Deflation	1.25 +/- 0.04	0.70 +/- 0.03	1.23 +/- 0.03	0.74 +/- 0.03
5th of Deflation	1.16 +/- 0.03	0.82 +/- 0.04	1.10 +/- 0.03	0.84 +/- 0.05
6th of Deflation	1.15 +/- 0.03	0.84 +/- 0.04	1.08 +/- 0.03	0.86 +/- 0.03

Table 3-4: Normalised values of Ti and Te during deflation to 5cm of water pressure in control and emphysematous rats.

	Control rats n=28 Deflation tests		Emphysematous rats n=28 Deflation tests	
Normalised value	Ti Deflation / Eupnoeic Ti	Te Deflation / Eupnoeic Te	Ti Deflation / Eupnoeic Ti	Te Deflation / Eupnoeic Te
Breath Number				
1st of Deflation	1.27 +/- 0.05	0.73 +/- 0.04	1.20 +/- 0.06	1.08 +/- 0.22
5th of Deflation	1.03 +/- 0.04	1.09 +/- 0.10	0.97 +/- 0.05	0.96 +/- 0.12
6th of Deflation	1.05 +/- 0.04	1.08 +/- 0.10	0.97 +/- 0.05	1.00 +/- 0.12

Table 3-5: Normalised value of Ti and Te during deflation to -10cm of water pressure in control and emphysematous rats.

The typical response to deflating the lungs was a reflex increase in T_i and shortening of T_e ; however not all rats from either group responded in this 'typical' manner. Some rats responded to deflation with little change in T_i or with some shortening of T_i followed by a reduced T_e . A response with both T_i and T_e shortened was called a 'non- typical' response, as these responses were less common. In 11% of the tests on normal rats a 'non-typical' response was obtained to deflation and 89% in a typical manner. In the diseased rats 18.5% of the tests were non-typical.

Fourteen control rats, tested twice each for their response to a deflation pressure of 5cm of water, resulted in 89% of them responding with a marked increase in T_i and a corresponding significant decrease in T_e which continued throughout the deflation. In the remaining 11% of tests the rats responded non-typically as although they showed a decrease in T_e they did not increase their T_i in the first breath of deflation, and in some cases a slightly reduced T_i was noted in the first and subsequent breaths during lung deflation. In 18.5% of 28 deflation tests on 14 diseased rats a non-typical response was found. These rats responded as did the non-typical control rats, showing little change in T_i and a reduction in T_e . In one rat from each of the control and diseased groups, on deflation of the lungs, the animal increased both inspiratory and expiratory timing of breathing.

In 12% of deflation tests, on control rats at negative 10cm of water pressure, the rats showed a decrease in T_i with a reduction in T_e . The decrease in T_i and T_e in these rats continued for all the breaths analysed during deflation. The typical deflation response of an increased T_i and reduced T_e was not found in 35% of the 28 deflation tests carried out on 14 diseased rats. In 14% of the tests the rats responded by decreasing T_i and increasing T_e . Rats responded in a consistent manner on repeated tests separated by several minutes recovery. The decrease in T_i was quite marked in these animals as was the increase in T_e . In the other 21% of the non-typical responses to deflation both inspiratory and expiratory time decreased. In the first breath taken after the lungs were deflated both T_i and T_e decreased in 3 of the 14 rats.

Figures 3-17 & 3-18 show typical and non-typical responses respectively.

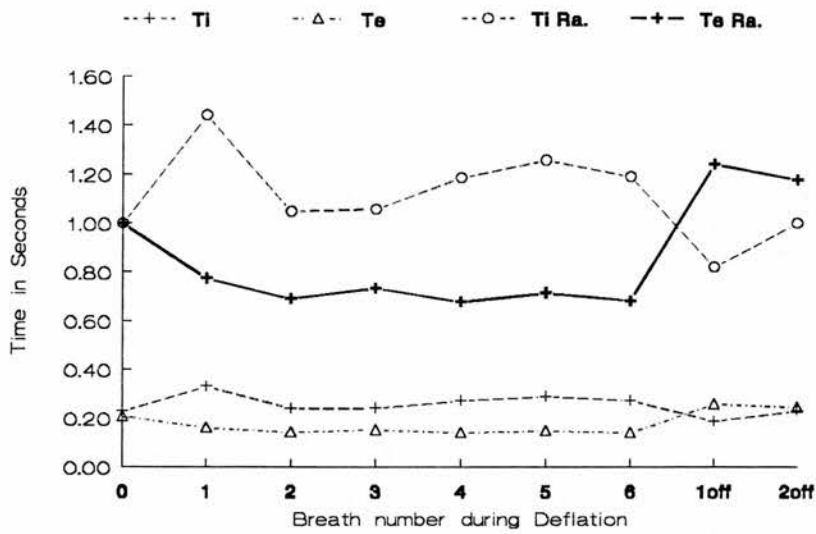


Figure 3-17: Typical response to deflation in a control rat to - 5cm of water pressure.

In this type of response Ti increases above the control value and Te decreases below the control value and they remain so for the period of the deflation.

The Ti and Te values for the control breath immediately before the deflation, (breath 0) breaths 1-6 during the deflation and the 1st and 2nd breaths immediately after the deflation, (1off and 2off) are shown. The normalised values of Ti and Te during deflation are also shown as Ti Ra and Te Ra respectively. The breath immediately before deflation has a normalised value of 1.

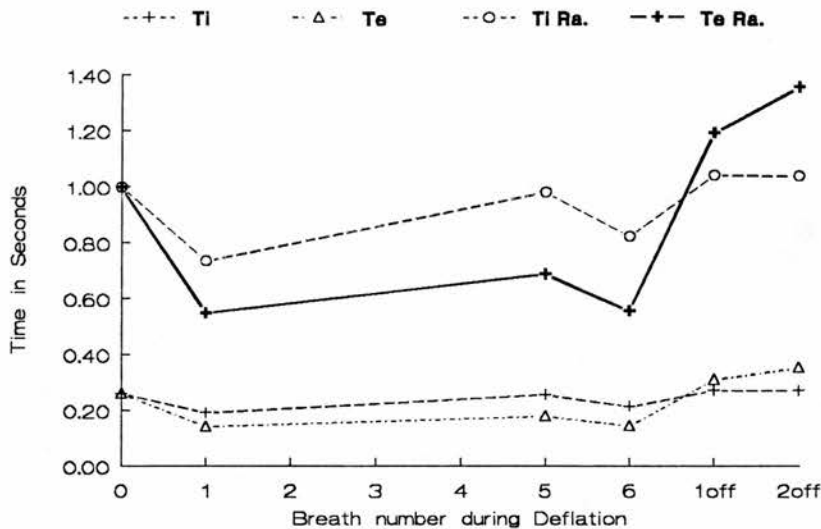


Figure 3-18: *Non-typical response to deflation in a control rat to - 10cm of water pressure.*

In this type of response both Ti and Te decrease below the control value and they remain so for the period of the deflation.

The Ti and Te values for the control breath immediately before the deflation, (breath 0) breaths 1-6 during the deflation and the 1st and 2nd breaths immediately after the deflation, (1off and 2off) are shown. The normalised values of Ti and Te during deflation are also shown as Ti Ra and Te Ra respectively. The breath immediately before deflation has a normalised value of 1.

	Control - 5cm H ₂ O	Diseased - 5cm H ₂ O	Control - 10cm H ₂ O	Diseased - 10cm H ₂ O
Ti increased Te decreased	89%	81.5%	88%	65%
Ti decreased Te decreased	11%	18.5%	12%	21%
Ti decreased Te increased	0%	0%	0%	14%

Table 3-6: *The proportion of the deflation tests in the control and diseased rats showing the pattern of the responses at different deflation pressures.*

BREATHING PATTERN OF RATS INHALING CARBON DIOXIDE.

In both the control and emphysematous rats the total breath time (Tt) did not alter significantly from the eupnoeic state when the rat breathed a gas mixture containing 4% carbon dioxide for a period of two minutes. The mean eupnoeic Tt for the control rats was 0.49 +/- 0.02s, (n=70, breaths 5 from each of 14 rats) and after 2 minutes 4% carbon dioxide Tt was 0.52 +/- 0.03s, (n=70, 5 breaths from each of 14 rats). In the 4 week diseased animals the mean eupnoeic Tt was 0.54 +/- 0.03s (n=90 breaths, 5 from each of 18 rats) and after 4% CO₂ Tt was 0.54 +/- 0.02s (n=90 breaths, 5 from each of 18 rats).

Breathing 6% CO₂ did not change Tt significantly. The control animals breathing 6% CO₂ for two minutes had a mean breath time of 0.520 +/- 0.03s (n=70) and the diseased animals had a mean breath time of 0.508 +/- 0.03s (n=90).

Comparing the total breath time for the control rats during 4% and 6% CO₂ with that of the diseased animals under the same conditions showed no significant difference between the two groups. The rats seemed to have very little response in terms of Tt to breathing carbon dioxide at the levels used.

Tidal volume was statistically significantly increased in both groups of rats when they were given air containing 4% and 6% carbon dioxide. The percentage increase in tidal volume was calculated at both levels of carbon dioxide and the results are shown in Table 3-7. The tidal volume, when breathing CO₂, was calculated in 11 control rats

and 12 diseased rats. Five breaths from each rat were analysed, and the expiratory tidal volume was calculated from the integral of expiratory flow for each of these breaths. The mean tidal volume for each rat was calculated as the average value of V_T for the five breaths. Tidal volume was very consistent between breaths in an individual rat. In the diseased group the tidal volume of one of the rats was reduced by breathing 4% and 6% CO_2 .

Carbon dioxide level	Control rats % change in V_T	Emphysematous rats % change in V_T
4%	135.25 +/- 6.03	124.10 +/- 4.56
6%	162 +/- 13.74 *	132.5 +/- 6.50

Table 3-7: *Percentage change in tidal volume for the control and emphysematous rats when breathing mixtures of air containing 4% and 6% CO_2 .*

** represents a statistically significant difference between the means ($P < 0.05$).*

The percentage change in V_T was greater in the control rats than in the diseased rats. The change was not statistically significant at the 4% level of CO_2 but was statistically significant at the 6% level of CO_2 when tested with the Student's t test.

CHAPTER 4

RECEPTOR ACTIVITY IN EUPNOEA.

CONDUCTION VELOCITIES.

Conduction velocities of stretch receptors and rapidly adapting receptors were measured as described in the Methods chapter. The receptors identified as stretch receptors had a mean conduction velocity of 36.13 ± 2.50 m/sec, ($n=8$) and the receptors identified as rapidly adapting had a mean conduction velocity of 14.5 ± 2.33 m/sec, ($n=4$). The mean values for conduction velocities of the two types of receptor were statistically different from each other ($P<0.001$).

RECEPTOR TYPES.

The activity of 85 vagal lung receptors was recorded from single afferent fibres of the left vagus nerve in control rats. Fibres could be divided into those displaying mainly inspiratory activity and those that displayed mainly expiratory activity. These corresponded exactly to Slowly Adapting or Rapidly Adapting receptors on the criterion of Adaptation Index described in *Methods*. The activity in 99 vagal fibres was recorded from the 1 week diseased rats and another 99 fibres were recorded from the rats that had been left for 4 weeks between Papain administration and receptor recording. These fibres from both groups of diseased rats could be clearly divided into fibres originating from the mainly inspiratory firing slowly adapting receptors (SARs), and the mainly expiratory firing rapidly adapting receptors (RARs). All had the characteristics of receptors with myelinated fibres when their discharge was inspected on an oscilloscope. No more than 5 fibres in each group of rats were found with discharge characteristics associated with unmyelinated fibres, (low discharge rate, low spike height compared to background and little correlation with the phases of breathing). These were abandoned.

RECEPTOR PROPORTIONS.

The proportion of inspiratory to expiratory firing types of receptors was the same for the control, the 1 week emphysematous and 4 week emphysematous rats. In the emphysematous rats the same proportions of RARs to SARs were found one week and four weeks after Papain administration. Of the 85 receptors recorded from

the control rats, 67.1% of them were found to be SARs, and the remaining 32.9% were RARs. In the 1 week diseased rats 65.6% of the receptors were of the SAR type and 34.4% were of the RAR type. In the 4 week diseased rats the proportion of SARs was 67.7% and the remaining 32.3% fired mostly in expiration and were classified as RARs.

The results for the Papain treated rats that were recorded from after 1 week of administration are not presented further and all the emphysematous rats subsequently referred to are the Papain treated rats that were recorded from 4 weeks after the administration of the enzyme.

The SARs for both groups of rats could be subdivided into three types on the basis of their discharge pattern during an eupnoeic respiratory cycle of tidal volume 2.83 ± 0.20 ml for the controls and 2.85 ± 0.16 ml for the four week emphysematous. These were called Types 1, 2 and 3. Type 1 SARs had a low discharge frequency and discharged almost exclusively during mid and late inspiration. Type 2 SARs were placed in a separate category from Type 1 because they had a significantly higher discharge frequency and the discharge occurred in early expiration. Type 3 SARs discharged throughout inspiration and expiration and had a higher firing frequency than either Type 1 or Type 2.

Figures 4-1 to 4-4 show typical examples of each of the categories of pulmonary vagal afferent fibres in which activity was recorded. SARs, Types 1, 2, 3, (fired mainly in inspiration) and RARs, (fired mainly in expiration). Traces of the raw data are shown as they were displayed in the script written for the Spike2 analysis program. The activity of action potentials fired from the pulmonary receptors is shown on the upper traces and flow rate of breathing (V) is shown on the lower traces. The frequency of individual action potentials (instantaneous firing frequency) is given in Hertz and the flow rate is given in litres per minute.

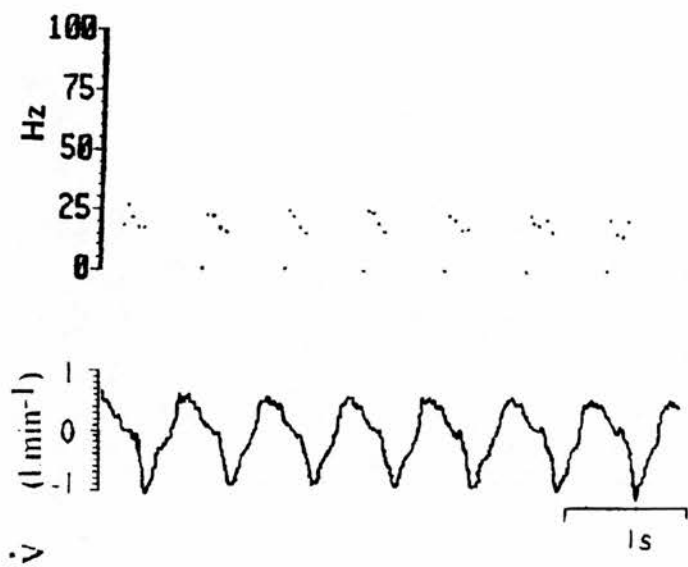


Figure 4-1: *Typical Type 1 slowly adapting receptor during eupnoeic breathing in controls.*

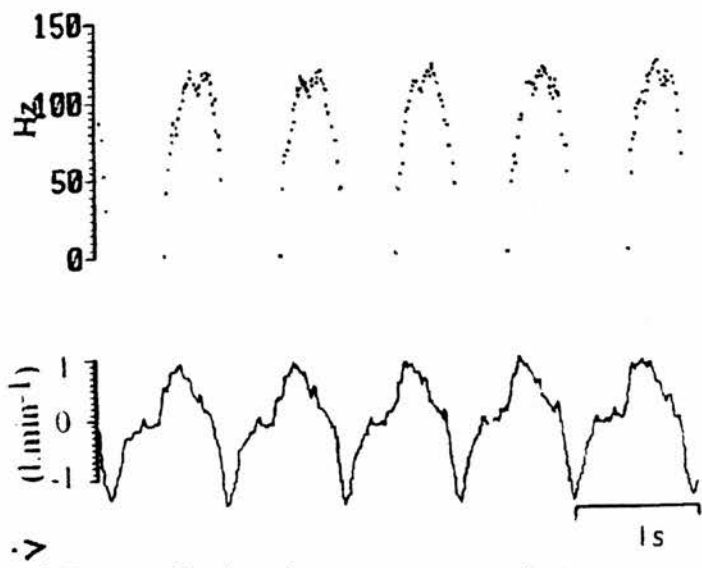


Figure 4-2: *Typical Type 2 slowly adapting receptor during eupnoeic breathing in controls.*

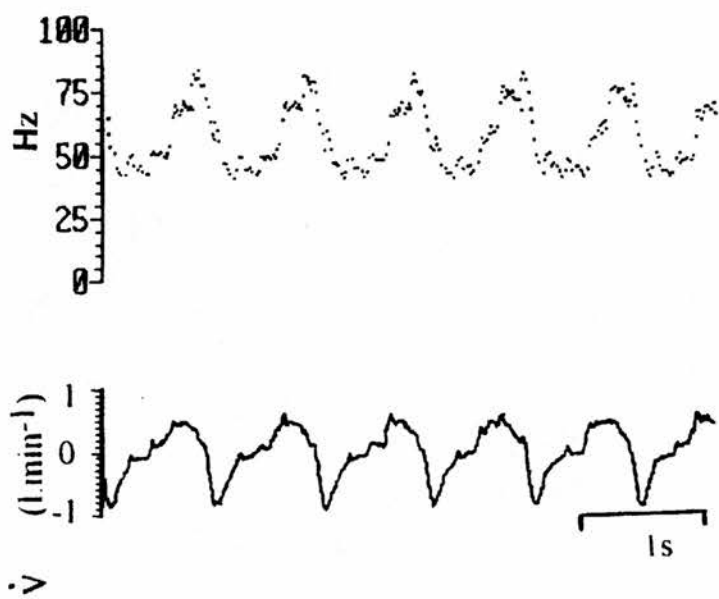


Figure 4-3: *Typical Type 3 slowly adapting receptor during eupnoeic breathing in controls.*

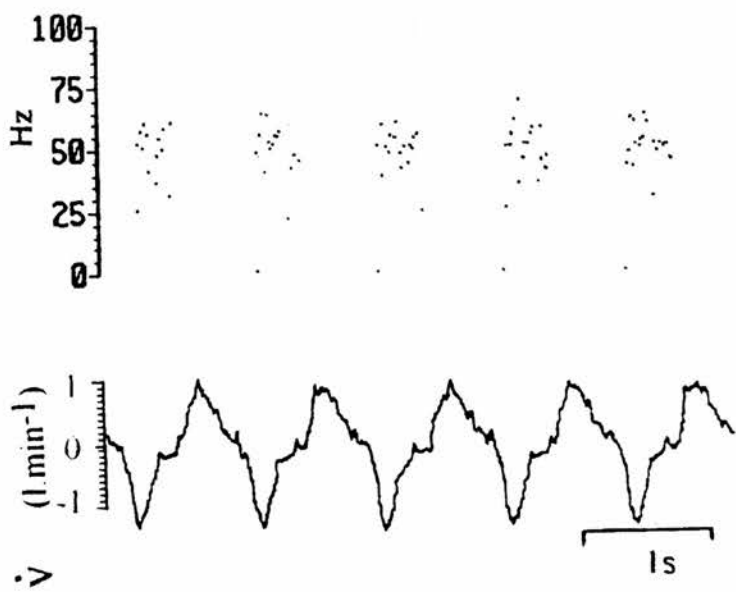


Figure 4-4: *Typical Rapidly adapting receptor during eupnoeic breathing in controls.*

The examples of receptors in Figures 4-1 to 4-4 are from control rats, however the receptors from the emphysematous rats were very similar. To illustrate this examples of a Type 2 SAR and a RAR from an emphysematous rat are given in Figures 4-5 and 4-6.

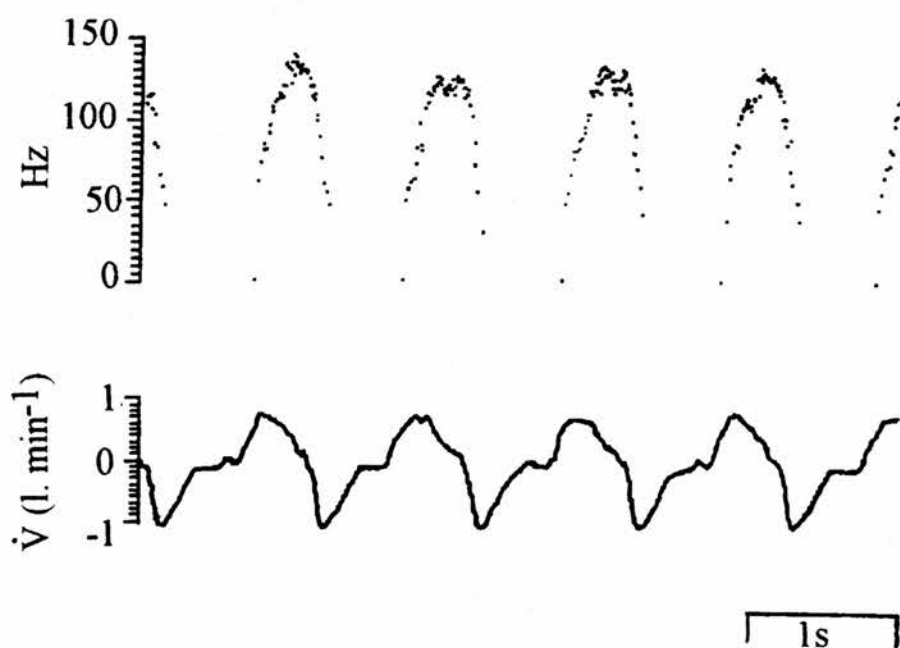


Figure 4-5: *Typical Type 2 slowly adapting receptor during eupnoeic breathing in emphysema.*

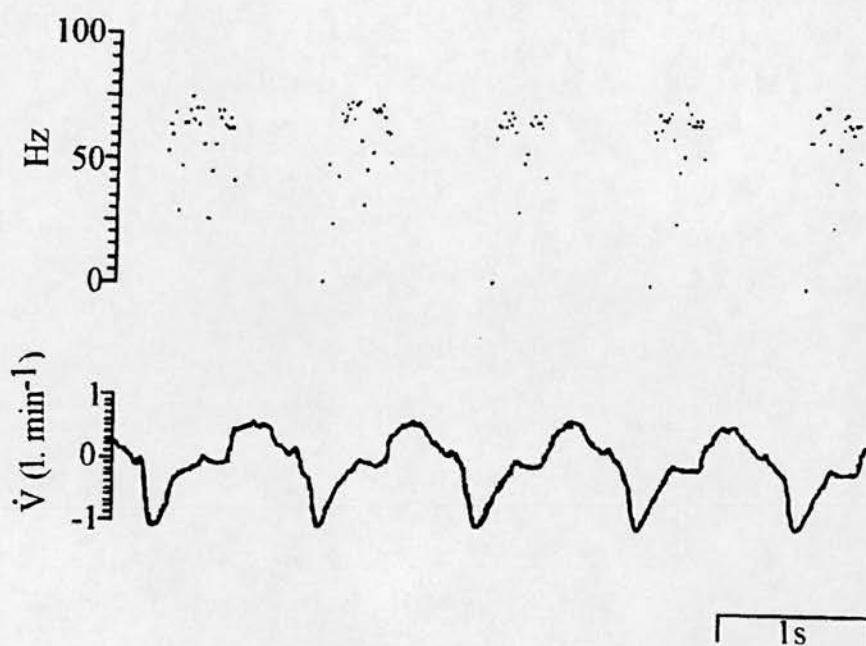


Figure 4-6: *Typical Rapidly adapting receptor from an emphysematous rat during eupnoeic breathing.*

Twelve of the 57 slowly adapting receptors, (21.0%) were classified as belonging to the Type 1 SAR group in the controls, while only 7 of the 67 SARs of the emphysematous rats, (10.5%) were classified as belonging to the Type 1 group. In the controls 30 of the SARs, (52.6%) were also classified as belonging to Type 2 and 44 of the 67, (65.7%) SARs in the diseased group were classified as belonging to this type. The remaining 15 SARs, (26.3%) in the control rats were classified as Type 3 receptors and 16, (23.9%) were of this type in the emphysematous rats. As a percentage of the total number of receptors including both SARs and RARs, 14.1%, 35.3% and 17.6% were classified as Types 1, 2 and 3 respectively in the control group and 7.1%, 44.4%, 16.2% were classified as Types 1, 2 and 3 respectively in the emphysematous group. These results are summarised in Tables 4-1 & 4-2.

Statistical analysis was performed on the actual numbers of the four different types of receptors in the two groups of rats. The analysis was performed to test if there was a statistical difference in the distribution of receptors between the different types in the diseased compared to the normal rats. The data was analysed using the chi - squared test. A chi - square value of 3.213 was calculated. At 3 degrees of freedom the probability that the two sets of data were from different populations was

well above the conventional level of 5%, ($0.50 > P > 0.10$). Therefore the null hypothesis was not disproved, showing there was no statistical significant difference in the distribution of the receptors among the four different types in the controls compared to the emphysematous rats.

A chi - squared test was also performed on the actual numbers of receptors in each of the three SAR sub-groups. This was carried out to find if the distribution of the SARs between the three sub-groups was statistically different in the controls and emphysematous rats. The chi - square test gave a value of 3.212, at 2 degrees of freedom giving a probability of $0.50 > P > 0.10$. Therefore there was no statistical significant difference between the two sets of data.

Receptor Type	Sub - Type	Control Rats	Diseased Rats
SAR	All	67.1%	67.7%
	Type 1	14.1%	7.1%
	Type 2	35.3%	44.4%
	Type 3	17.6%	16.2%
RAR		32.9%	32.3%

Table 4-1: *Proportions of the various types of receptors in the control and emphysematous rats, given as percentages of the total number of receptors that were recorded.*

Receptor Type	Control Rats	Diseased Rats
SAR Type 1	21.0%	10.5%
SAR Type 2	52.6%	65.7%
SAR Type 3	26.3%	23.9%

Table 4-2 *Proportion of the different sub types of the SARs given as percentages of the total number of SARs.*

Not all these receptors were suitable for further computer analysis although they could be categorised manually. The receptors that were not used for action potential analysis were found not to be single fibres when viewed carefully on the computer screen, although they had appeared to be so during the experiment when they were examined on the oscilloscope. Although the fibres were not single this did not represent a problem in determining receptor type. Further analysis was questionable as the computer could not discriminate between the action potentials arising from the two receptors when recorded on the same trace. Such fibres were abandoned.

In the controls 12 of Type 1 and only 27 of Type 2 and 11 of Type 3 were assessed as being suitable for the computerised action potential analysis, and in the emphysematous groups similar numbers of receptors were found to be unsuitable for further analysis. Five of Type 1, 40 of Type 2 and 13 of Type 3 were suitable.

Of the 28 RARs recorded from in the controls 22, were assessed as being suitable for further analysis. Twenty eight of the 32 RARs from the diseased rats were assessed as suitable for the detailed computer analysis.

SLOWLY ADAPTING STRETCH RECEPTORS DURING EUPNOEIC BREATHING.

CONSISTENCY OF DISCHARGE.

The SARs displayed a regular firing pattern which was highly consistent between breaths for a particular receptor. The number of action potentials firing in each phase of breathing was very regular for each breath. Five consecutive breaths during eupnoea were analysed for each receptor. The results of the number of action potentials occurring in each phase of breathing, for three receptors during five consecutive breaths, are given in Table 4-3. Data from one of each type of SAR, (Type 1, 2 and 3) is given in Table 4-3 to show that all types of SARs had a regular pattern of activity. Although only one example from each type is given these receptors are representative of their types and all the SARs recorded from were very constant in terms of the number of action potentials they fired per breath and per breath phase.

Receptor Type	Breath Number	No. of spikes per phase (Ti)	No. of spikes per phase (Te)
1	1	7	0
	2	9	0
	3	8	0
	4	8	0
	5	7	0
2	1	16	2
	2	17	2
	3	16	2
	4	17	1
	5	14	3
3	1	31	9
	2	30	10
	3	33	6
	4	31	9
	5	33	8

Table 4-3: *The number of action potentials occurring from three SARs in the control rats during five consecutive eupnoeic breaths.*

This consistent firing pattern was also displayed by the SARs of the emphysematous rats. All the SARs from both the controls and the diseased rats had a significantly higher peak, minimum and number of action potentials per second occurring during inspiration compared to expiration.

As well as consistency in the number of action potentials fired per phase of breathing, the position at which the action potentials occurred within the phase was also highly regular and consistent for an individual receptor. Figure 4-7 shows the percentage time of inspiration at which 25, 50, 75 and 100% of the total number of action potentials which fired in Ti occurred by. This is shown for two individual SARs, (Type 2) during 5 consecutive breaths.

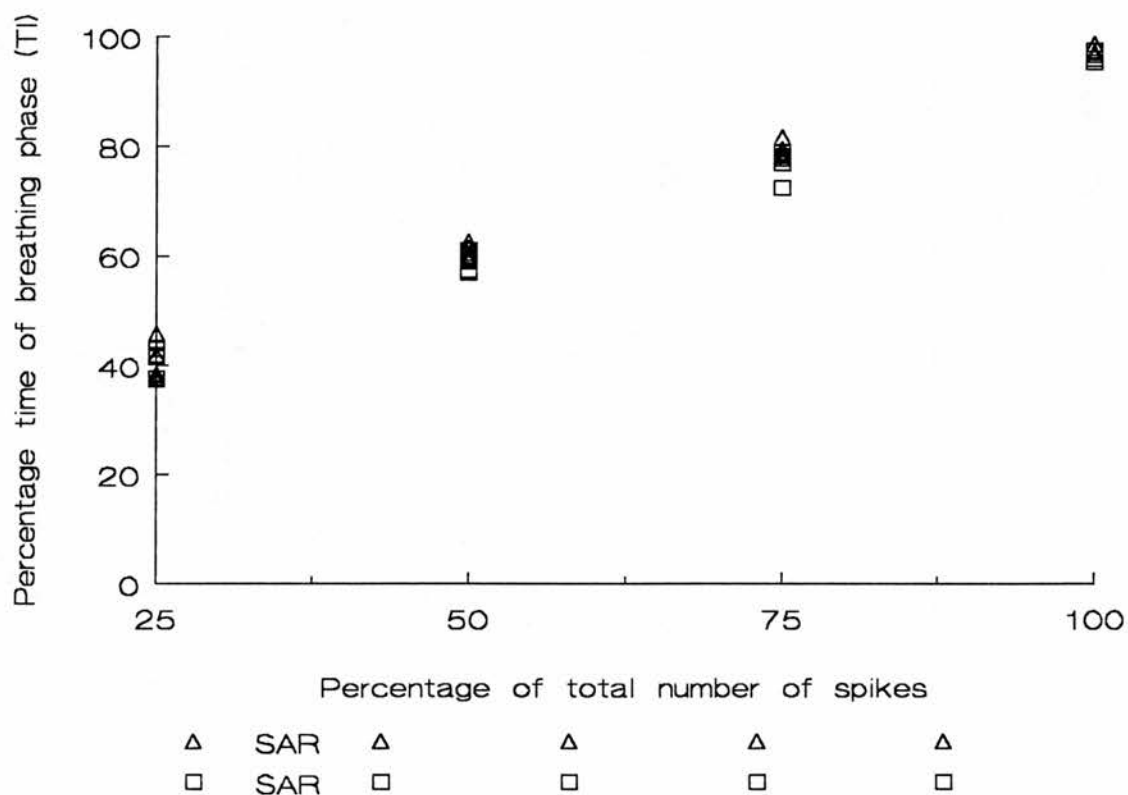


Figure 4-7: *Percentage time of inspiration at which 25, 50, 75 and 100% of the action potentials from two SARs occurred during five consecutive breaths.*

PATTERN OF DISCHARGE.

The mean value for the peak discharge frequency of all SARs was found. This was calculated from the minimum spike interval period between two action potentials within a breath phase. The peak firing frequency was measured during both Ti and Te for 5 consecutive breaths for each of the stretch receptors that were analysed. The average number of action potentials firing per second and the mean minimum frequency of the action potentials were also calculated during each phase of breathing. The number of action potentials firing within each Ti and Te of the breaths was also counted, (see Tables 4-4 & 4-5).

During Ti Phase of breathing	Control Rats	Emphysematous Rats
Mean Peak Frequency in HZ	86.1 +/- 1.98 *	91.2 +/- 1.98 *
Mean minimum Frequency in HZ	38.9 +/- 0.79	39.7 +/- 0.67
Number of Action Potentials / Second	57.4 +/- 1.69	58.3 +/- 1.33
Number of Action Potentials / Phase	22.4 +/- 0.71	20.2 +/- 0.56

Table 4-4: *Activity of SARs during inspiratory phase of eupnoeic breathing in control and emphysematous rats.*

Each receptor was analysed for 5 consecutive eupnoeic breaths.

* Statistically significant difference between control and emphysematous ($P < 0.05$)

n = 250 breaths for the controls (50 SARs)

n = 290 breaths for the emphysematous group (58 SARs).

During Te Phase of breathing	Control Rats	Emphysematous Rats
Mean Peak Frequency in HZ	57.1 +/- 2.40 *	64.8 +/- 2.37 *
Mean minimum Frequency in HZ	25.7 +/- 1.32	27.6 +/- 1.26
Number of Action Potentials / Second	13.2 +/- 0.99	14.3 +/- 0.80
Number of Action Potentials / Phase	6.0 +/- 0.40	6.0 +/- 0.30

Table 4-5: *Activity of SARs during expiratory phase of eupnoeic breathing in control and emphysematous rats.*

Each receptor analysed for 5 consecutive eupnoeic breaths.

* Statistically significant difference between control and emphysematous ($P < 0.05$)

n = 250 breaths for controls (50 SARs).

n = 290 breaths for the emphysematous group (58 SARs).

SARs had a higher maximum frequency in the diseased animals during both Ti and Te than did those in the control rats. The mean peak firing frequency in the diseased rats was found to be significantly different to the mean peak firing frequency of the controls at $P < 0.05$. The diseased rats also had an increased number of action potentials occurring per second during both Ti and Te, although the differences were found to not reach significance. The minimum frequency of action potentials firing in Ti and Te were also found to be slightly higher in the diseased rats, however this was not found to represent a significant difference between the means when tested with the Student's t- test.

The results showing the activity of the three types of SARs during both inspiration and expiration are shown in the Tables App.4-1 to App.4-6 (see Appendix 4).

RELATIONSHIP BETWEEN NEURO-PHYSIOLOGICAL CHANGES AND THE SEVERITY OF THE DISEASE.

Neuro-physiological data was examined to assess if changes in the activity of the vagal lung receptors could be related to the severity of the disease as determined by the volume pressure relations.

The relationships between changes in neuro-physiological activity and severity of the disease as assessed from the compliance measurements was investigated in four rats for which both neuro-physiological and compliance data was available. The severity of the emphysema in the rats ranged from a rat with a high lung compliance of 1.0 ml/cm.H₂O, indicating that the rat had severe emphysema to a rat with a relatively low compliance of 0.46 ml/cm.H₂O in which the emphysema was relatively mild. The other two papain treated rats had lung compliance values between these two extremes, as shown in Table 4-6.

RAT	Compliance ml/cm.H ₂ O	No. of Type1 SARs	No. of Type2 SARs	No. of Type3 SARs	No. of RARs
1	0.46	0	2	0	1
2	0.56	0	2	0	2
3	0.61	1	2	0	2
4	1.0	0	1	4	0

Table 4-6: *The different types of receptors that were recorded from in the Papain treated rats in which static lung compliance was measured.*

HERING-BREUER REFLEX STRENGTH RELATED TO THE SEVERITY OF THE EMPHYSEMA.

In the vagally intact state the Hering-Breuer pause of the rats was measured using an inflation pressure of 5cm of water. The Hering-Breuer ratio was calculated for each animal by dividing the actual Hering-Breuer pause by the previous eupnoeic expiratory duration. The strength of the Hering-Breuer reflex was related to the severity of the disease in terms of compliance. Figure 4-8 shows the relationship between the strength of the Hering-Breuer reflex and the severity of the disease.

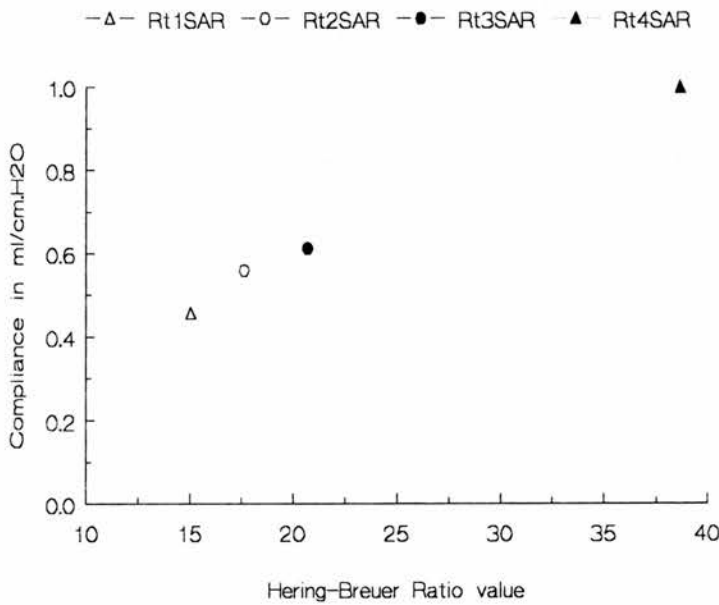


Figure 4-8: Relationship between compliance and Hering-Breuer ratio value for four papain treated rats.

The results show a positive correlation between lung compliance and strength of the Hering-Breuer inflation reflex. Spearman's rank correlation value was calculated to be 1. However the statistical significance of this correlation value was not tested. This was decided after consultation with a medical statistician who advised that any test for significance would be unreliable as the sample size was so small.

Despite this the results still suggest a positive correlation between lung compliance and Hering-Breuer pause ratio, i.e. the higher the lung compliance and therefore the more severe the emphysema, the longer the Hering-Breuer pause.

The available correlation data between severity of the disease and strength of the Hering-Breuer pause shows that the animals with the more severe emphysema have longer Hering-Breuer ratios. This could be due to the increase in SAR activity as the severity of the disease increases.

ACTIVITY OF SLOWLY ADAPTING RECEPTORS DURING EUPNOEA IN RELATION TO THE SEVERITY OF THE DISEASE.

In each Papain treated rat for which pressure / volume relations were recorded the activity of a slowly adapting receptor, firing mainly in inspiration, was examined. The average maximum frequency of each receptor was calculated over five consecutive breaths during the inspiratory phase. The static lung compliance for an individual rat was related to the activity of one SAR in terms of maximum firing frequency. This relationship was plotted for four papain treated rats for which static lung compliance data was available, see Figure 4-9. Slowly adapting Type 2 receptors were examined to investigate the relationship between activity of receptors and severity of the disease, as this type of receptor had been recorded in each of the rats in which lung compliance data was available. Data was not available in all the compliance measured rats for the other types of receptors.

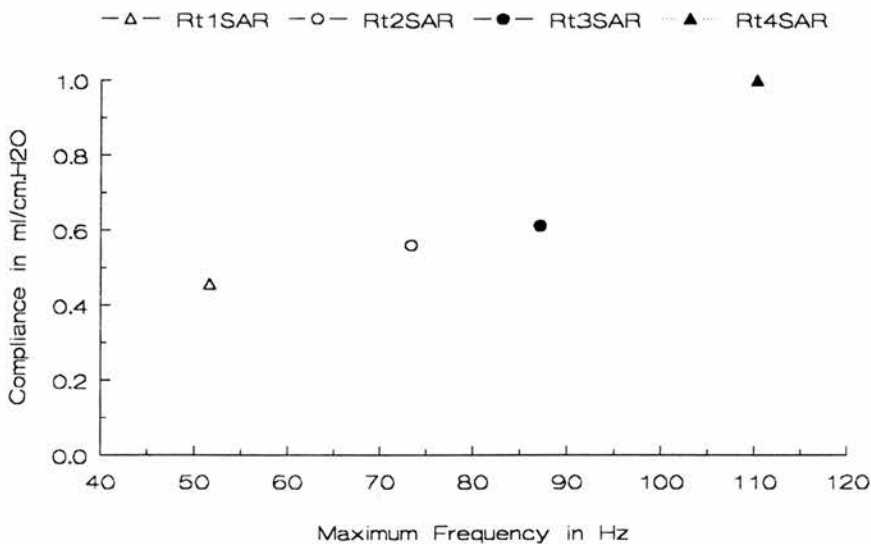


Figure 4-9: Relationship between lung compliance and the maximum firing frequency of a typical SAR. One slowly adapting receptor (Type 2 in each instance) was examined from each of the rats for which compliance data was available.

The results show that there is a positive correlation between the lung compliance and the maximum firing frequency of SARs in the emphysematous rats during eupnoeic breathing. Spearman's rank correlation was calculated to be 1. However the statistical significance of this correlation could not be tested reliably due to the very low value for n.

ACTIVITY OF SLOWLY ADAPTING RECEPTORS ON LUNG INFLATION IN RELATION TO THE SEVERITY OF DISEASE.

The activity of slowly adapting vagal lung receptors was analysed in the rats for which the severity of the disease was known in terms of lung compliance. Activity of SARs was compared during the first and third 0.25s interval from when an inflation pressure of 5cm.H₂O was applied. The calculation was as follows :-

$$\frac{\text{Number of A.Ps in 1st 0.25s interval of inflation} - \text{Number of A.Ps in 3rd 0.25s interval}}{\text{Number of A.Ps in the 1st 0.25s interval}}$$

This value was then multiplied by a hundred to give a percentage value of the SAR's adaptation to inflation. The activity that remained after 0.75 seconds of inflation was then calculated. The relationship between lung compliance and the percentage of SAR activity remaining after 0.75 seconds of inflation was then plotted. This relationship was first established for the Type 2 receptors previously analysed for their maximum frequency during eupnoeic breathing, (see Figure 4-10) and then for all of the SARs recorded from in the four compliance measured emphysematous rats, (Figure 4-11).

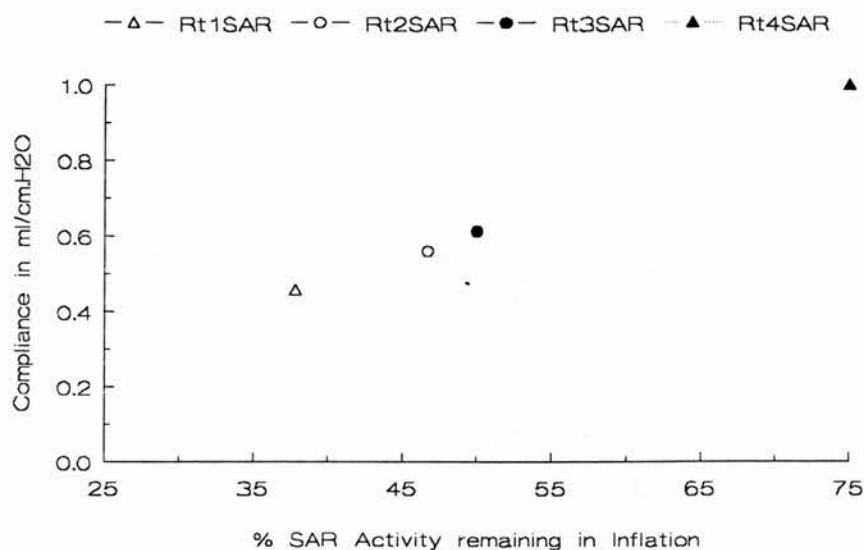


Figure 4-10: *Relationship between lung compliance and the adaptation of SARs during lung inflation to 5cm of water pressure in emphysematous rats. One slowly adapting receptor (Type 2 in each instance) was examined from each of the rats for which compliance data was available.*

Figure 4-10 shows a positive correlation between the severity of the disease in terms of compliance and the percentage of activity remaining in the SARs. Spearman's rank correlation was 1, again however the statistical significance of this value could not be tested reliably.

The results however are suggestive that a positive correlation exists between lung compliance and percentage of activity remaining in the Type 2 SARs after lung inflation. Therefore this indicates that a negative correlation exists between lung compliance and adaptation of SARs to lung inflation. The more severely diseased rats have SARs that adapt less to inflation and therefore maintain a higher firing frequency during inflation than do receptors from less severely diseased rats, as shown in Table 4-7.

RAT	Lung Compliance ml/cm.H ₂ O o	Mean % Adaptation of all SARs	Mean % Activity remaining in all SARs
1	0.46	57.55	42.45
2	0.56	50.75	49.25
3	0.61	52.65	47.35
4	1.0	26.74	73.26

Table 4-7: Table to show the mean percentage adaptation in all the SARs of the lung compliance measured emphysematous rats after 0.75 seconds of inflation.

The most diseased rat had SARs that adapted less to lung inflation than did the receptors from the less severely diseased rats. That is, the overall SAR activity in the most severely diseased rat remained at a higher level during lung inflation than it did in the not so diseased animals.

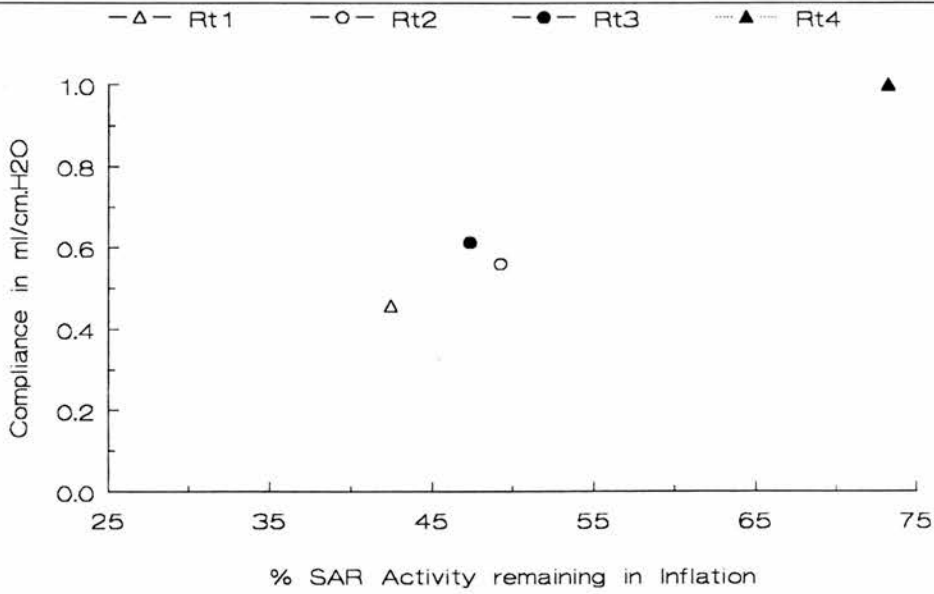


Figure 4-11: Relationship between lung compliance and the adaptation of SARs during lung inflation to 5cm of water pressure in emphysematous rats, for all the SARs recorded from in the compliance measured rats.

Figure 4-11 shows a relationship between the severity of the disease and the activity of SARs during lung inflation. Spearman's rank correlation was calculated to be 0.8, statistical significance of this value was not calculated as this relationship was only investigated for a limited number of receptors. However the results are still suggestive that the severity of the disease might be related to the receptor activity and the degree of adaptation of the receptors during lung inflation.

In summary, the Hering-Breuer inflation reflex, the maximum firing frequency during eupnoea and the SARs' lower adaptation to lung inflation all appear to have a relationship to the severity of emphysema found in the individual rats in which the SARs were recorded.

There were positive correlations between severity of the disease and strength of the Hering-Breuer reflex, activity of SARs during eupnoea, activity of Type 2 SARs on lung inflation and activity of all the SARs on lung inflation. The Hering-Breuer reflex tended to be longer as the severity of the disease increased. The average maximum firing frequency tended to increase with the severity of the disease and the adaptation of the SARs to lung inflation decreased as the severity of the emphysema increased. However, for these results to be confirmed the activity of a much larger number of receptors should be related to the severity of the emphysema in the animals in which they are recorded from.

The relationship between neuro-physiological changes and the severity of the disease needs to be determined in a larger group of animals. This would allow the statistical significance of any correlations to be determined. The correlations between neuro-physiological changes and the severity of the emphysema found in the present investigation require confirmation from further studies recording the activity of receptors in many more animals in which the severity of the disease is known.

RAPIDLY ADAPTING RECEPTORS DURING EUPNOEIC BREATHING.

CONSISTENCY OF DISCHARGE.

The firing pattern was less regular than that of the SARs but for an individual RAR the number of action potentials occurring from breath to breath was highly consistent.

Receptor Number	Breath Number	No. of spikes per phase (Ti)	No. of spikes per phase (Te)
1	1	0	24
	2	0	22
	3	0	22
	4	0	21
	5	0	22
2	1	2	14
	2	2	14
	3	2	12
	4	1	13
	5	1	13
3	1	1	6
	2	1	5
	3	2	7
	4	1	5
	5	1	6

Table 4-8: *The number of action potentials occurring from three RARs in the control rats during five consecutive eupnoeic breaths.*

The three receptors chosen for illustration show the range of firing frequencies displayed by the RARs in the control rats.

Not only was the number of action potentials fired per phase consistent but also the position in a breath at which they occurred was also highly regular for a particular receptor. Figure 4-12 shows the percentage time of Te at which 25, 50, 75 and 100% of the total discharge occurred for 2 typical RARs over 5 consecutive breaths.

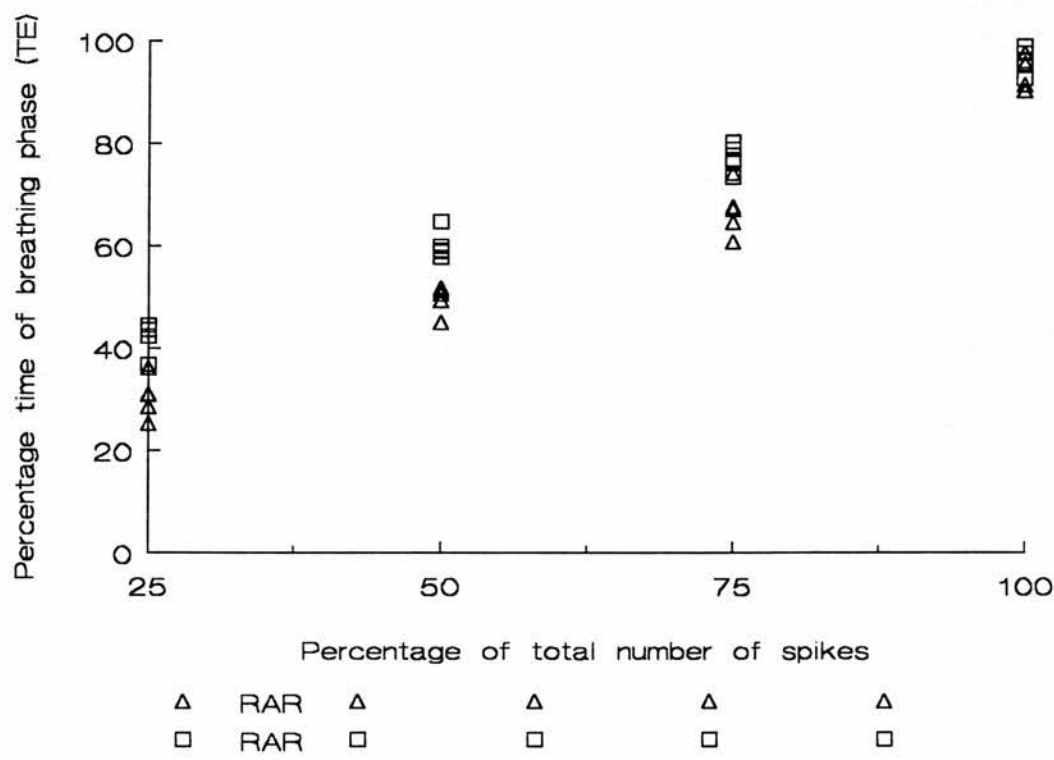


Figure 4-12: *Percentage time of Te at which 25, 50, 75 and 100% of the action potentials from two RARs occurred during five consecutive breaths.*

PATTERN OF DISCHARGE.

The activity of the RARs in the control and diseased rats was analysed during eupnoeic breathing. The activity of these mainly expiratory firing receptors was measured during both inspiration and expiration as for the mainly inspiratory firing SARs.

The RARs of both groups of rats were found to have a statistically significant greater mean peak firing frequency, number of action potentials per second, number of action potentials per phase and mean minimum frequency during expiration than during inspiration.

The RARs fired very consistently during eupnoea, in terms of the number of action potentials fired per breath and per phase of breathing during consecutive eupnoeic breaths.

In the control rats the number of action potentials firing in inspiration was quite low ranging from 0-11, while during expiration the firing ranged from 2-28 action potentials per phase. In the diseased rats a similar range of number of action potentials per breath phase was found; 0-8 during inspiration and 3-29 in expiration.

In expiration a statistically significant greater mean peak firing frequency, number of action potentials firing per second and number of action potentials occurring per phase of breathing was found in the RARs of the emphysematous rats compared to those in the controls. No differences in RAR firing frequency or number of action potentials occurring per phase was found between the diseased and control groups during the inspiratory phase. Tables 4-9 and 4-10 show the discharge characteristics of 22 RARs from the control rats and 28 RARs from the diseased rats during eupnoeic inspiration and expiration respectively.

During Ti Phase of breathing	Control Rats (n=110)	Emphysematous Rats (n=140)
Mean Peak Frequency in HZ	18.5 +/- 3.00	18.0 +/- 2.46
Mean minimum Frequency in HZ	7.7 +/- 1.20	8.6 +/- 1.23
Number of Action Potentials / second	5.0 +/- 0.55	4.8 +/- 0.55
Number of Action Potentials / phase	2.6 +/- 0.23	2.1 +/- 0.32

Table 4-9: *Activity of RARs during inspiratory phase of eupnoeic breathing in control and emphysematous rats.*

Each receptor analysed for 5 consecutive eupnoeic breaths.

During Te Phase of breathing	Control Rats (n=110)	Emphysematous Rats (n=140)
Mean Peak Frequency in HZ	87.5 +/- 6.10	118.8 +/- 5.71***
Mean minimum Frequency in HZ	18.1 +/- 1.08	18.8 +/- 1.04
Number of Action Potentials / second	28.8 +/- 1.25	34.6 +/- 1.25**
Number of Action potentials / phase	13.3 +/- 0.55	15.1 +/- 0.58*

Table 4-10: *Activity of RARs during expiratory phase of eupnoeic breathing in control and emphysematous rats.*

Each receptor analysed for 5 consecutive eupnoeic breaths.

*** represents a statistically significant difference between the control and emphysematous rats ($P < 0.001$).

** represents a statistically significant difference between the control and emphysematous rats ($P < 0.01$).

* represents a statistically significant difference between the control and emphysematous rats ($P < 0.05$).

DISTRIBUTION OF ACTION POTENTIAL DISCHARGE DURING EUPNOEIC BREATHS.

Figure 4-13 shows the distribution of action potential discharge within a breath for all the receptors analysed in the control rats. This analysis was done in the control rats in order to define the normal position at which action potentials fired within a breath. The four different types of receptors were analysed to define where they fired within a breath in the normal animals.

For each receptor the time at which every action potential occurred during inspiration and expiration was calculated in terms of the percentage time of the respective breathing phase, as described in the methods data analysis section.

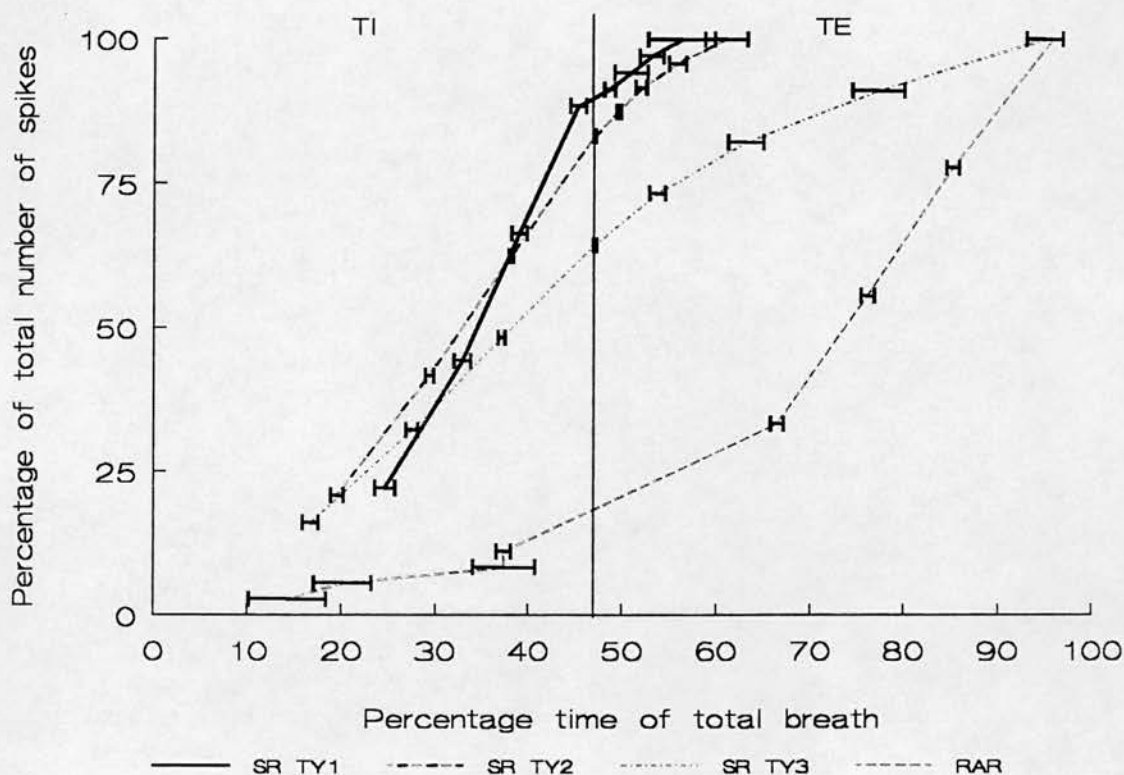


Figure 4-13: *Percentage time of total breath at which action potentials from pulmonary receptors in control rats occur in terms of the % of the total number of action potentials that fire within a breath.*

SLOWLY ADAPTING STRETCH RECEPTORS.

TYPE 1.

The Type 1 receptors fired mainly within the inspiratory phase. For 12 Type 1 receptors analysed, 25%, 50%, 75% and 100% of the number of action potentials in inspiration occurred at mean percentage times of $51.34 \pm 1.07\%$, $67.34 \pm 0.88\%$, $81.30 \pm 0.83\%$ and $94.37 \pm 0.83\%$ of T_i respectively, ($n=12$). Only a few receptors of the Type 1 displayed any activity during expiration. Of the action potentials from Type 1 receptors which did occur in expiration 25%, 50%, 75% and 100% occurred at mean percentage times of $1.28 \pm 0.58\%$, $5.60 \pm 1.77\%$, $9.81 \pm 1.26\%$ and

15.87 \pm 1.55% of Te respectively. Thus these receptors discharged mainly during mid and late inspiration, as shown in Figure 4-1.

TYPE 2.

These receptors discharged throughout inspiration and early expiration as shown in Figure 4-2. For 27 Type 2 receptors 25%, 50%, 75% and 100% of the number of action potentials occurring in Ti occurred at mean percentage times of 40.68 \pm 0.68%, 61.17 \pm 0.43%, 79.48 \pm 0.23% and 98.10 \pm 0.13% of Ti respectively. During expiration their discharge was restricted to the first quarter of Te. Twenty five percent, 50%, 75% and 100% of all action potentials in that phase occurred at mean percentage times of 3.11 \pm 0.22%, 7.67 \pm 0.57%, 14.99 \pm 0.90% and 25.00 \pm 2.27% of Te respectively. Thus these receptors discharged over more of the breathing cycle than did the Type 1 receptors. The Type 2 receptors were placed in a separate category to the Type 1 because of their lower adaptation index and significantly higher discharge frequency during both inspiration and expiration compared to the Type 1 receptors.

TYPE 3.

These receptors discharged throughout inspiration and expiration as shown in Figure 4-3. Eleven Type 3 receptors were analysed. Twenty five percent, 50%, 75% and 100% of the action potentials from these receptors that fired during inspiration occurred at mean percentage times of 34.84 \pm 0.84%, 57.33 \pm 0.62%, 77.35 \pm 0.37% and 98.11 \pm 0.17% of Ti respectively. During expiration their discharge extended throughout the phase with 25%, 50%, 75% and 100% of all spikes in expiration occurring at mean percentage times of 11.00 \pm 0.85%, 29.03 \pm 1.88%, 56.26 \pm 2.80% and 90.34 \pm 1.93% of Te respectively.

RAPIDLY ADAPTING RECEPTORS.

These discharged mostly during expiration in eupnoea as shown in Figure 4-4. During expiration 25%, 50%, 75% and 100% of the spikes occurred by 35.5 \pm 0.77%, 54.10 \pm 0.67%, 71.65 \pm 0.73% and 92.07 \pm 0.69% of Te respectively, (n=110 breaths, 5 from each of 22 RARs). Only a few action potentials from the RARs fired during inspiration. Of the action potentials from Rapidly adapting receptors which did occur in inspiration 25%, 50%, 75% and 100% occurred at mean percentage times of 29.69 \pm 4.12 %, 41.99 \pm 3.06 %, 77.94 \pm 2.07 % and 70.66 \pm 3.3 % of Ti respectively.

CHAPTER 5

RECEPTOR RESPONSE TO LUNG INFLATION.

SLOWLY ADAPTING STRETCH RECEPTORS.

The SARs of both the control and the emphysematous rats were stimulated by lung inflations of 5 and 10cm of water pressure.

Figures 5-1 to 5-3 and Figures 5-4 to 5-6 show control SAR Types 1, 2 and 3 responses to inflation pressures of 5cm and 10cm of water respectively. The illustrations are examples of SARs from control rats, however the responses of SARs from the emphysematous rats to these inflation pressures were very similar in their characteristics see Figure 5-7.

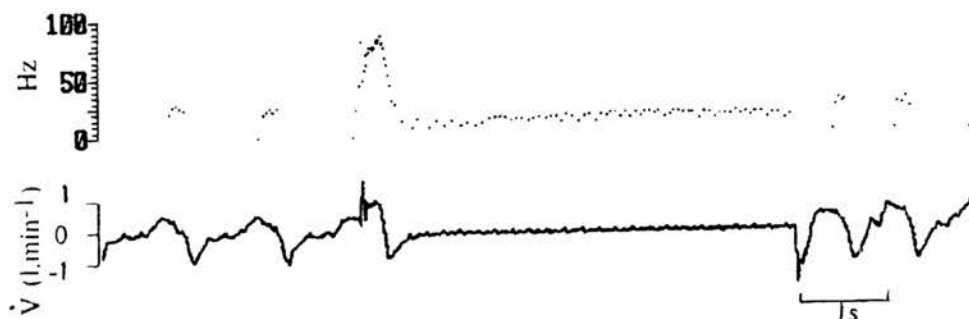


Figure 5-1: *Response of a typical SAR Type 1 from a control rat to an inflation pressure of 5 cm of water.*

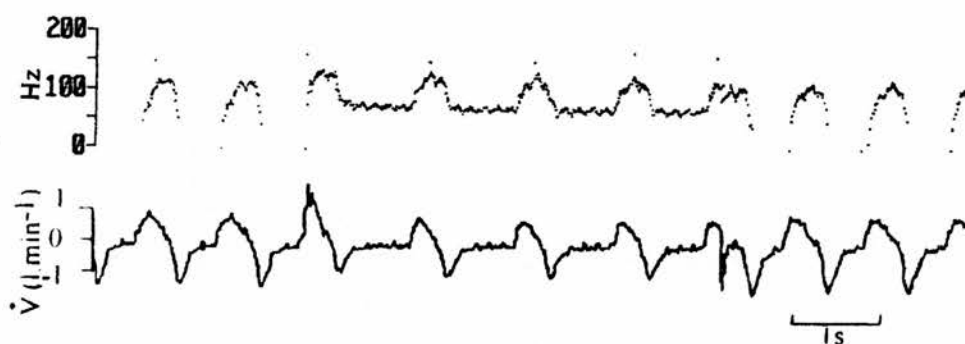


Figure 5-2: Response of a typical SAR Type 2 from a control rat to an inflation pressure of 5 cm of water.

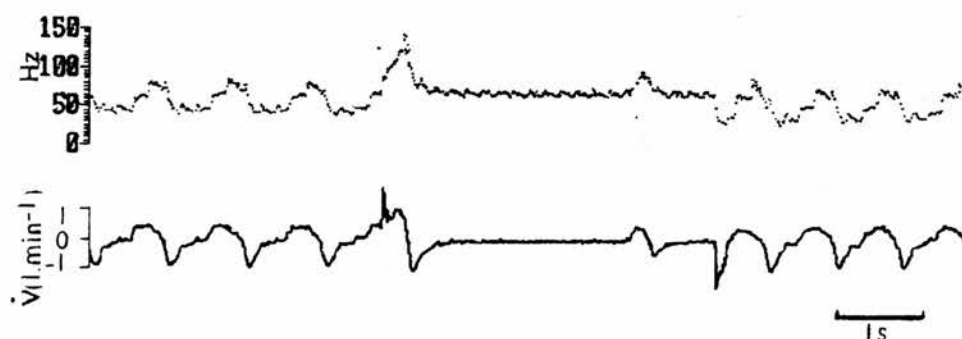


Figure 5-3: Response of a typical SAR Type 3 from a control rat to an inflation pressure of 5 cm of water.

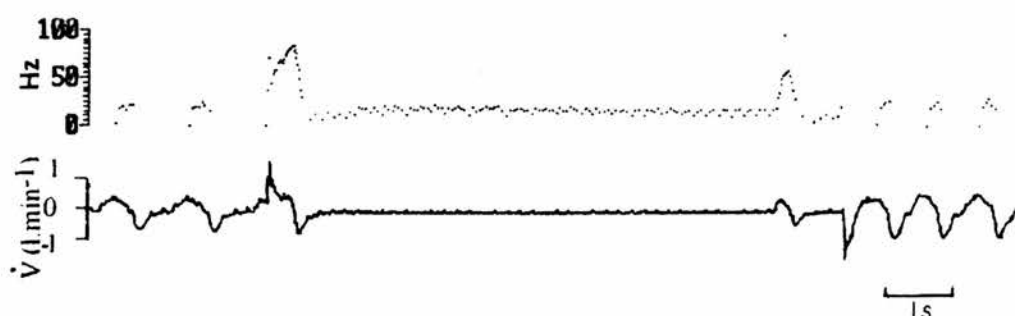


Figure 5-4: Response of a typical SAR Type 1 from a control rat to an inflation pressure of 10 cm of water.

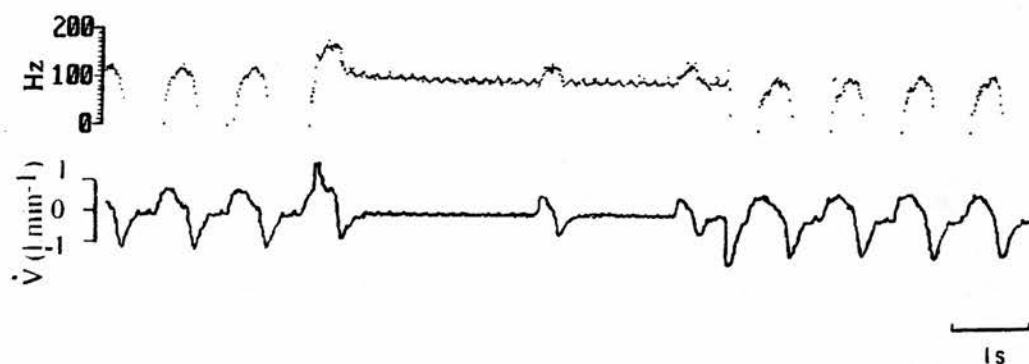


Figure 5-5: Response of a typical SAR Type 2 from a control rat to an inflation pressure of 10 cm of water.

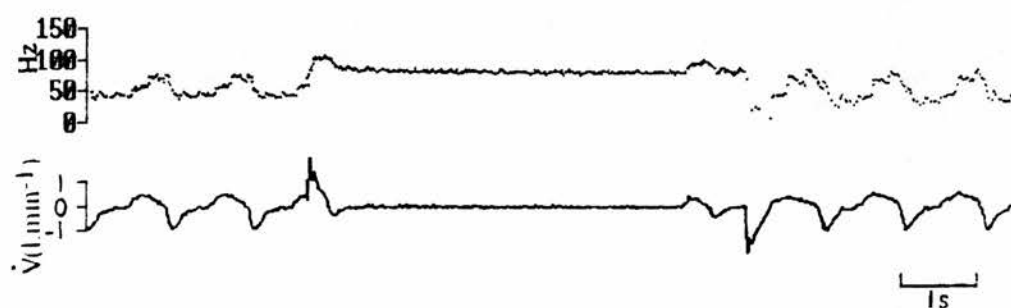


Figure 5-6: Response of a typical SAR Type 3 from a control rat to an inflation pressure of 10 cm of water.

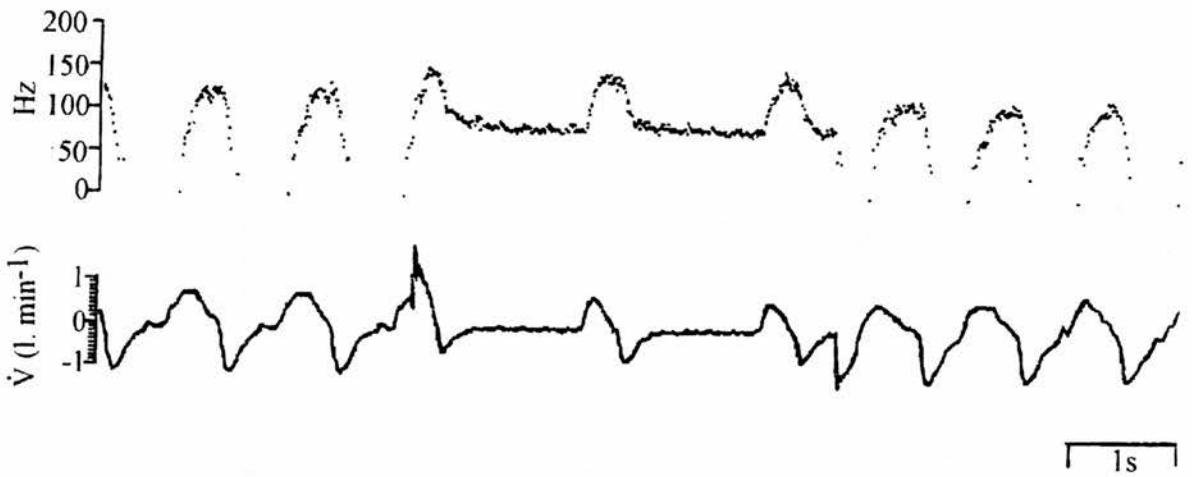


Figure 5-7: Response of a typical SAR Type 2 from an emphysematous rat to an inflation pressure of 5 cm of water.

The increased stimulation of the receptors by inflation of the lungs is shown in Table 5-1. Table 5-1 also shows that the receptors were stimulated more by pressures of 10cm of water than by a pressures of 5cm of water. The number of action potentials occurring per second during the inflation was compared to the number of impulses occurring during the immediately preceding eupnoeic inspiration. This greater activity of the SARs of both groups of rats during inflation, measured as an increase in the number of action potentials occurring per second, was found to represent a statistically significant increase in activity using the Student's t-test at $P < 0.01$.

	Inflation pressure 5cm of water No. of impulses per Sec		Inflation pressure 10cm of water No. of impulses per Sec	
	Ti of Breath before inflation	During 1st 0.25s of inflation	Ti of Breath before inflation	During 1st 0.25s of inflation
Controls	56.85 +/- 3.83	103.31 +/- 4.64	55.72 +/- 3.72	115.24 +/- 4.62
Emphysematous	58.44 +/- 3.11	112.74 +/- 4.74	56.58 +/- 2.94	121.61 +/- 4.14

Table 5-1: *Number of action potentials per second arising from SARs of control and emphysematous rats during the inspiration immediately preceding inflation and during the first 0.25s interval of inflation to 5 and 10cm of water pressure.*

49 SARs in the control rats were tested with inflation at 5 and 10cm of water pressure.

52 SARs in the emphysematous rats were tested with inflation at 5 and 10cm of water pressure.

The number of action potentials occurring per second during the inflation was greater in the emphysematous rats than in the controls, both at 5 and 10cm of water pressure; however these differences were not statistically significant when tested with the Student's t-test.

The control rats increased their SAR activity in terms of number of action potentials occurring per second by 182% and 206% at 5cm and 10cm of water pressure respectively. The emphysematous rats increased their SAR activity by 193% and 215% at 5 and 10cm of water pressure respectively.

Peak frequency of the SARs from both groups of rats was also greater during inflation than during eupnoea. Peak frequency was not used as an indication of activity during inflation as the opening of the electromagnetic valves, which applied inflation, produced an electrical pulse which confounded this aspect of the analysis.

ADAPTATION OF THE SLOWLY ADAPTING STRETCH RECEPTORS.

When the rats' lungs were inflated with a positive pressure of 5cm of water, SARs of both the control and diseased animals adapted relatively slowly. The controls had a mean adaptation index of $60.82 \pm 3.33\%$, ($n=49$) for all their SARs analysed, including 11 of Type 1, 27 of Type 2 and 11 of Type 3. The diseased rats had a statistically significantly lower adaptation index of $49.95 \pm 2.21\%$; ($n=52$) for their SARs, including 5 of Type 1, 34 of Type 2 and 13 of Type 3. This lower adaptation index of SARs from the emphysematous rats was statistically significantly different from the adaptation index of the SARs from the control rats at $P < 0.01$, (See Table 5-2).

Inflation Pressure	n	Control Rats % Adaptation	Emphysematous Rats % Adaptation
+ 5cm H ₂ O	49	$60.82 \pm 3.33^{**}$	49.95 ± 2.21
+ 10cm H ₂ O	52	$37.96 \pm 2.59^{*}$	31.05 ± 2.16

Table 5-2: *Adaptation index of control and emphysematous SARs to inflation pressures of 5 and 10cm of water.*

** represents a statistically significant difference between the adaptation of SARs from the control and emphysematous rats to inflation ($P < 0.01$).

* represents a statistically significant difference between the adaptation of SARs from the control and emphysematous rats to inflation ($P < 0.05$)

In the control rats the mean adaptation index of all the SARs to an inflation pressure of 10cm of water pressure was $37.96 \pm 2.59\%$, ($n=49$, Type 1 - 11, Type 2 - 27, Type 3 - 11), while in the diseased rats the SARs had a mean adaptation index of $31.05 \pm 2.16\%$, ($n=52$, Type 1 - 5, Type 2 - 34, Type 3 - 13) at the same inflation pressure. Six of the emphysematous rats' Type 2 receptors were unsuitable for analysis during inflation. The emphysematous rats showed a statistically significantly lower mean adaptation to inflation of 10cm of water pressure at $P < 0.05$ compared to the control rats. As the SARs from the diseased animals had a lower mean adaptation

index the increased activity of these receptors, due to inflation, persisted longer in the emphysematous rats than in the controls.

The mean adaptation index of the different categories of SARs was calculated. The Type 1 receptors of the control rats had a mean adaptation index of 58.23 +/- 7.82%, (n=11). (The value of n is one less than in the eupnoeic state as one of the fibres from a Type 1 SAR was lost during the experiment after eupnoeic breathing had been collected but before lung inflation could be applied to the rat). In the emphysematous rats the mean adaptation index of Type 1 receptors was 50.48 +/- 7.74, (n=5). The lower adaptation index of the Type 1 receptors of the emphysematous rats was not found to be significantly different when they were compared with the Type 1 receptors of the control rats independently from the other inspiratory receptors.

Type 2 receptors in the control rats had a mean adaptation index of 37.04 +/- 2.71%, (n=27) and in the diseased rats 31.30 +/- 1.86%, (n=34). This difference was just beyond statistical significance.

The Type 3 receptors in the controls had a mean adaptation index of 29.39 +/- 4.68%, (n=11) and the diseased 22.92 +/- 3.43%, (n=13). This was found not to represent a statistically significant difference; although again as with the other types of receptors the SARs of the diseased rats adapted less to inflation.

The mean adaptation indexes of the three types of SARs at 5 and 10cm of water pressure are given in Tables 5-3 and 5-4, for the control and emphysematous rats respectively.

Type of SAR	% adaptation to inflation 5cm H ₂ O	% adaptation to inflation 10cm H ₂ O	n
1	82.39 +/- 7.08	58.23 +/- 7.82	11
2	58.67 +/- 3.89	37.04 +/- 2.71	27
3	44.11 +/- 3.13	29.39 +/- 3.81	11

Table 5-3: *Adaptation indexes of SARs in the Control rats.*

Type of SAR	% adaptation to inflation 5cm H ₂ O	% adaptation to inflation 10cm H ₂ O	n
1	72.16 +/- 0.77	50.48 +/- 7.74	5
2	52.63 +/- 2.26	31.30 +/- 1.86	34
3	43.15 +/- 4.95	22.92 +/- 3.43	13

Table 5-4: *Adaptation indexes of SARs in the emphysematous rats.*

SARs of the emphysematous rats had a lower adaptation index compared to those of the control rats. The activity of the SARs was analysed in terms of the number of action potentials occurring per 0.25 second intervals from the onset of inflation. The results are shown in Tables 5-6 and 5-7 for the control and diseased animals at 5 and 10cm of water pressure respectively.

Receptor Type	Time from onset of Inflation	Number of Impulses in Control	Number of Impulses in Emphysema
1	1st 0.25 sec	16.6 +/- 1.30	13.8 +/- 2.13
	3rd 0.25 sec	3.18 +/- 1.33	4.0 +/- 1.87
2	1st 0.25 sec	27.63 +/- 1.30	29.0 +/- 1.15
	3rd 0.25 sec	11.38 +/- 1.12	13.40 +/- 0.69
3	1st 0.25 sec	31.0 +/- 2.12	33.39 +/- 1.99
	3rd 0.25 sec	17.09 +/- 1.25	19.31 +/- 2.00

Table 5-6: *Number of impulses from SARs of control and emphysematous rats that occurred in the first and third 0.25 seconds after the inflation pressure of 5cm of water was applied.*

Activity in 1st and 3rd 0.25 seconds during inflation given for SARs Types 1, 2 and 3. For the number of receptors of each type analysed, see the text.

Receptor Type	Time from onset of Inflation	Number of Impulses in Control	Number of Impulses in Emphysema
1	1st 0.25 sec	19.4 +/- 1.84	19.4 +/- 2.58
	3rd 0.25 sec	8.9 +/- 1.85	9.6 +/- 1.81
2	1st 0.25 sec	30.25 +/- 1.13	29.79 +/- 1.20
	3rd 0.25 sec	19.0 +/- 0.81	19.61 +/- 0.58
3	1st 0.25 sec	34.0 +/- 2.32	34.14 +/- 1.81
	3rd 0.25 sec	24.8 +/- 2.08	25.86 +/- 2.08

Table 5-7: Number of impulses from SARs of control and emphysematous rats that occurred in the first and third 0.25 seconds after the inflation pressure of 10cm of water was applied.

Activity in 1st and 3rd 0.25 second during inflation given for SARs Types 1, 2 and 3. For the number of receptors of each type analysed, see the text.

RAPIDLY ADAPTING RECEPTORS.

The expiratory receptors of both groups of rats responded to inflation pressures of 5 and 10cm of water with a brief stimulation in activity that adapted rapidly to the inflation. The RARs stopped firing completely within 0.25s. Figures 5-8 & 5-9 show typical responses of a RAR to inflation of the lungs at 5 and 10cm of water pressure respectively. Although the examples in Figures 5-8 & 5-9 are of a RAR from a control rat the RARs from the diseased rats responded in a similar manner see Figure 5-10 of a RAR from an emphysematous rat at an inflation pressure of 5cm of water pressure.



Figure 5-8: *Response of a typical RAR from a control rat to a lung inflation of 5 cm of water pressure.*

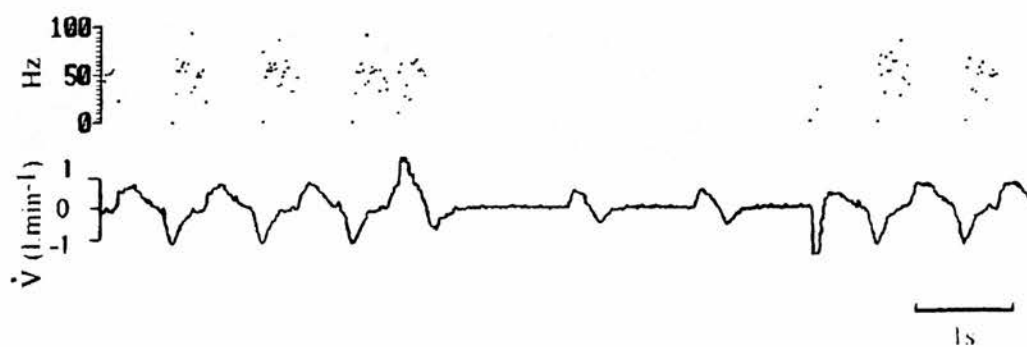


Figure 5-9: *Response of a typical RAR from a control rat to a lung inflation of 10 cm of water pressure.*

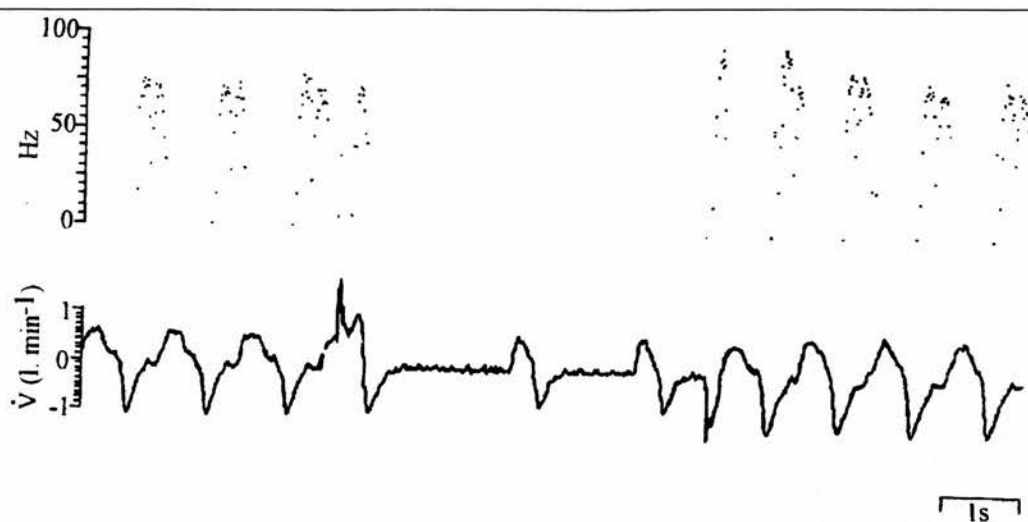


Figure 5-10: *Response of a typical RAR from an emphysematous rat to a lung inflation of 5 cm of water pressure.*

ADAPTATION OF THE EXPIRATORY FIRING RECEPTORS.

Rapidly adapting receptors had an index of 100% in both the control and the diseased rats.

CHAPTER 6

RECEPTOR RESPONSES TO DEFLATION OF THE LUNGS.

RAPIDLY ADAPTING RECEPTORS.

The expiratory firing rapidly adapting receptors of both groups of rats were stimulated by deflation of the lungs by 5 and 10cm of water pressure. There was an increase in the firing per phase and per second during the deflation compared to eupnoeic breathing. The activity of the receptors was measured during a eupnoeic breath immediately before the deflation and during the first, fifth and sixth breath of the deflation, as described in the Methods chapter. Figures 6-1 & 6-2 show a typical response of a RAR to a deflation pressure of 5 and 10cm of water pressure respectively. Although the RAR shown in these figures is from a control rat, the RARs from the emphysematous rats behaved similarly see Figure 6-3. In Figures 6-1 to 6-3 flow is displayed in litres per minute on the lower trace and the frequency of the action potentials fired from the receptor (instantaneous firing frequency of the receptors) is displayed in HZ on the upper trace.

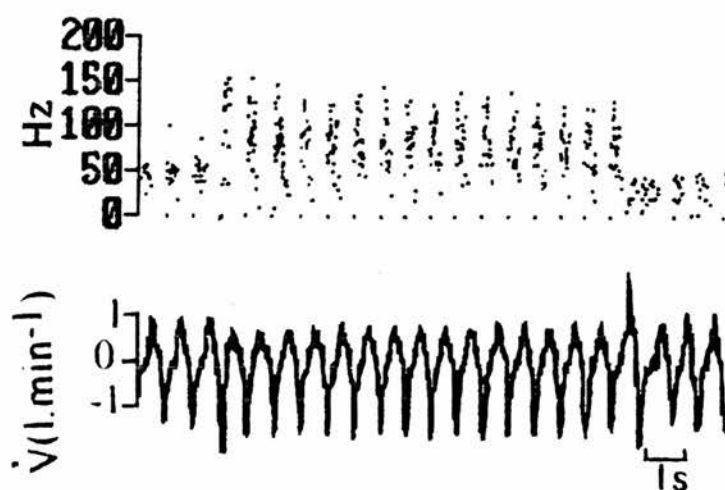


Figure 6-1: *Response of a typical rapidly adapting receptor from a control rat to a deflation pressure of 5 cm of water.*

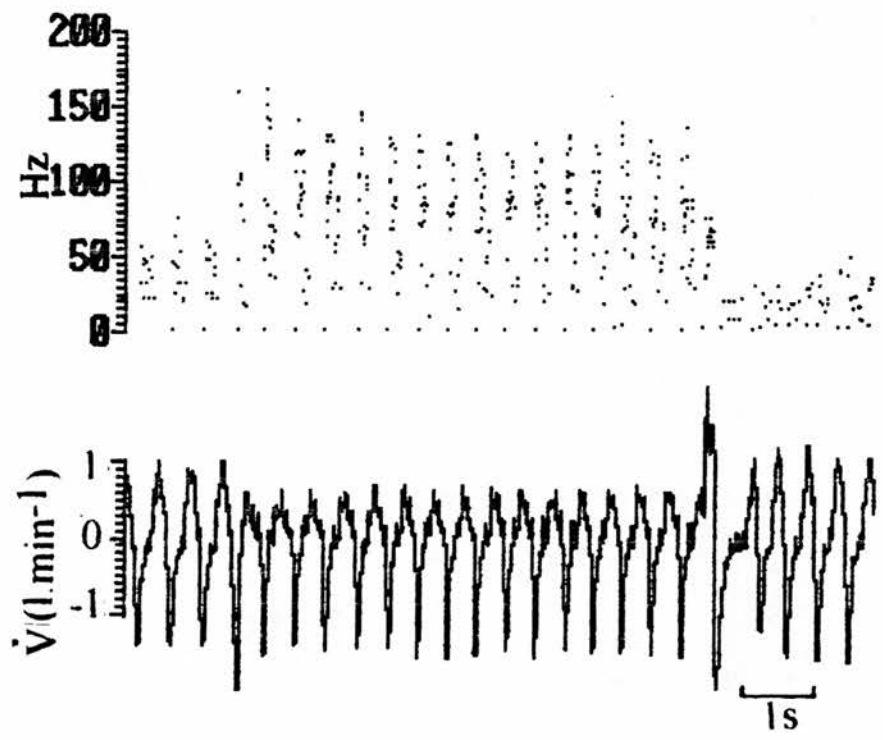


Figure 6-2: *Response of a typical rapidly adapting receptor from a control rat to a deflation pressure of 10cm of water.*

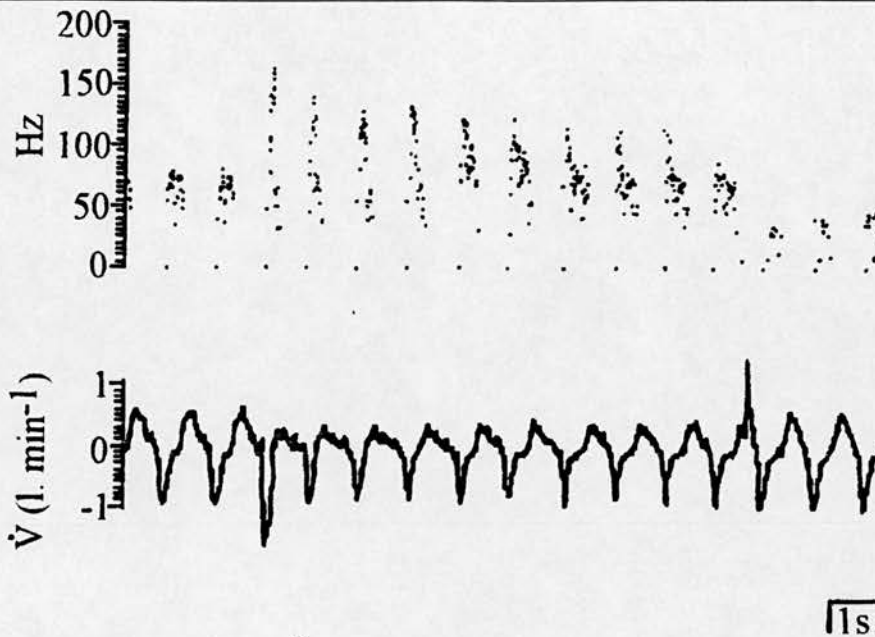


Figure 6-3: *Response of a typical rapidly adapting receptor from an emphysematous rat to a deflation pressure of 5cm of water.*

Eighteen RARs from control rats and 24 RARs from emphysematous rats were analysed during deflation. Tables 6-1 to 6-6 show the activity of the RARs during lung deflation compared to a control breath immediately before deflation. The response of the RARs to lung deflation of 5cm.H₂O are given in Tables 6-1 to 6-3, while the RARs response to deflations of 10cm.H₂O is given in Tables 6-3 to 6-6.

The RARs from both groups of rats showed an increase in activity on lung deflation which remained throughout the period of deflation. At the two deflation pressures of 5 and 10cm of water in both the control and emphysematous rats the number of action potentials arising from the RARs in Ti and Te increased when the lungs were deflated, and remained at an increased level throughout the deflation, see Tables 6-1 and 6-4. Activity of the RARs from both the control and emphysematous rats increased on lung deflation in terms of number of action potentials per phase, see Tables 6-1 and 6-4; number of action potentials per second, see Tables 6-2 and 6.5 and mean maximum firing frequency, see Table 6-3 and 6-6.

The increased activity of the RARs seen during deflation was compared between the control and diseased groups, to identify if either group increased the activity of their RARs more than the other. At the deflation pressure of 5cm.H₂O the RARs of the two groups of rats responded similarly in terms of number of action potentials per phase. However in terms of number of action potentials per second and

mean maximum firing frequency the RARs of the emphysematous rats were more active than those of the controls, see Tables 6-2 and 6-3 respectively.

In the first breath after the deflation of 10cm of water was applied no difference between the control and diseased rats was found in the number of action potentials occurring in the inspiration and expiration. By the fifth breath the control rats showed a significantly greater number of action potentials occurring during expiration compared to the emphysematous group, see Table 6-4. In the sixth breath the controls still showed a greater activity than the diseased, but this did not reach statistical significance.

When the activity of the receptors was analysed in terms of the number of action potentials occurring per second at 10cm.H₂O the control rats had a slightly higher firing frequency than the diseased rats during deflation, but this did not reach statistical significance when tested by the Student's t-test, see Table 6-5. The RARs of the controls were also slightly more active than those of the emphysematous rats in terms of mean maximum firing frequency, but this also did not reach statistical significance when tested by the Student's t-test.

Breath Number	Control rats Number of action potentials occurring in		Emphysematous rats Number of action potentials occurring in	
	Ti	Te	Ti	Te
Control breath	1.40 +/- 0.32	13.22 +/- 1.89	1.09 +/- 0.35	13.35 +/- 1.50
1st breath	2.61 +/- 1.20	19.45 +/- 2.41	2.52 +/- 0.56	23.04 +/- 2.67
5th breath	2.45 +/- 1.10	24.00 +/- 2.76	2.52 +/- 0.59	24.17 +/- 2.70
6th breath	1.94 +/- 0.60	25.67 +/- 3.13	2.35 +/- 0.54	23.56 +/- 2.61

Table 6-1: *Number of action potentials occurring per respiratory phase during deflation of the lungs to 5cm of water pressure and during the immediately preceding eupnoeic breath.*

Responses to deflation of 18 RARs from controls and 24 RARs from emphysematous rats.

Breath Number	Control rats Number of action potentials occurring per second in		Emphysematous rats Number of action potentials occurring per second in	
	Ti	Te	Ti	Te
Control breath	3.62 +/- 0.89	27.67 +/- 3.58	2.68 +/- 0.83	28.89 +/- 3.26
1st breath	5.80 +/- 2.55	62.74 +/- 6.90	6.04 +/- 1.33	71.23 +/- 6.08
5th breath	7.63 +/- 4.21	60.23 +/- 6.34	6.21 +/- 1.46	62.10 +/- 5.59
6th breath	5.03 +/- 1.44	61.95 +/- 6.51	6.15 +/- 1.44	64.23 +/- 5.32

Table 6-2: *Number of action potentials occurring per second during deflation of the lungs to 5cm of water pressure and during the immediately preceding eupnoeic breath.*

Responses to deflation of 18 RARs from controls and 24 RARs from emphysematous rats.

Breath Number	Control rats mean Maximum Firing Frequency Hz		Emphysematous rats mean Maximum Firing Frequency Hz	
	Ti	Te	Ti	Te
Control breath	10.33 +/- 3.77	73.80* +/- 9.13	7.14 +/- 2.49	105.11 +/- 12.30
1st breath	20.48 +/- 10.68	166.50 +/- 24.65	40.15 +/- 10.65	180.27 +/- 12.04
5th breath	21.62 +/- 10.92	153.51 +/- 13.97	40.12 +/- 11.39	160.71 +/- 14.77
6th breath	22.73 +/- 8.28	150.39 +/- 16.92	39.73 +/- 10.74	173.92 +/- 14.33

Table 6-3: *Maximum firing frequency of RARs during deflation of the lungs to 5cm of water pressure and during the immediately preceding eupnoeic breath.*

* Statistically significant difference between the mean values of maximum firing frequency of RARs in the control and diseased rats during the eupnoeic breath ($P < 0.05$).

Responses to deflation of 18 RARs from controls and 24 RARs from emphysematous rats.

Breath Number	Control rats Number of action potentials occurring in		Emphysematous rats Number of action potentials occurring in	
	Ti	Te	Ti	Te
Control breath	2.22 +/- 0.54	12.33 +/- 2.05	1.45 +/- 0.44	13.38 +/- 1.46
1st breath	3.39 +/- 0.97	21.72 +/- 2.71	4.88 +/- 0.93	26.88 +/- 3.69
5th breath	4.94 +/- 1.28	35.0* +/- 4.83	4.67 +/- 0.95	23.92 +/- 3.09
6th breath	4.61 +/- 1.19	31.6 +/- 4.24	5.83 +/- 1.41	25.26 +/- 3.27

Table 6-4: *Number of RAR action potentials occurring per respiratory phase during deflation of the lungs to 10cm of water pressure and during the immediately preceding eupnoeic breath.*

* Control rat RARs during the fifth breath had a statistically significantly greater number of action potentials in Te ($P < 0.05$).

Responses to deflation of 18 RARs from controls and 24 RARs from emphysematous rats.

Breath Number	Control rats Number of action potentials occurring per second in		Emphysematous rats Number of action potentials occurring per second in	
	Ti	Te	Ti	Te
Control breath	6.32 +/- 1.68	24.84 +/- 3.83	3.93 +/- 1.07	30.45 +/- 3.02
1st breath	9.14 +/- 2.83	68.25 +/- 7.69	14.51 +/- 2.91	63.06 +/- 5.60
5th breath	13.52 +/- 3.53	56.63 +/- 6.66	12.46 +/- 2.94	54.04 +/- 5.92
6th breath	12.52 +/- 3.43	54.47 +/- 6.51	16.36 +/- 3.78	59.30 +/- 7.77

Table 6-5: *Number of action potentials occurring per second during deflation of the lungs to 10cm of water pressure and during the immediately preceding eupnoeic breath.*

Responses to deflation of 18 RARs from controls and 24 RARs from emphysematous rats.

Breath Number	Control rats mean Maximum Firing Frequency Hz		Emphysematous rats mean Maximum Firing Frequency Hz	
	Ti	Te	Ti	Te
Control breath	20.28 +/- 5.69	65.05* +/- 9.43	14.91 +/- 5.53	99.74 +/- 9.52
1st breath	39.23 +/- 15.12	188.80 +/- 26.98	61.40 +/- 12.25	164.52 +/- 11.94
5th breath	50.02 +/- 12.78	152.79 +/- 17.73	58.11 +/- 10.69	151.48 +/- 18.07
6th breath	47.50 +/- 14.64	161.26 +/- 25.55	49.70 +/- 10.82	145.06 +/- 16.73

Table 6-6: *Maximum firing frequency of RARs during deflation of the lungs to 10cm of water pressure and during the immediately preceding eupnoeic breath.*

* represents a statistically significant difference between the maximum frequency of RARs in the control and diseased rats during the eupnoeic breath ($P < 0.05$).

Responses to deflation of 18 RARs from controls and 24 RARs from emphysematous rats.

SLOWLY ADAPTING RECEPTORS.

All SARs from both groups of rats reduced their activity when a deflation pressure of either 5 or 10cm of water was applied. Figures 6-4 to 6-9 illustrate the responses of SARs Types 1, 2 and 3 from control rats to deflation pressures of 5 and 10cm of water pressure. Although the illustrations are examples from control rats the reduced activity on deflation was very similar in the SARs of diseased rats see Figure 6-10 of a Type 2 SAR from an emphysematous rat. In each figure, flow is displayed in litres per minute on the lower trace and the frequency of the action potentials fired from the receptors are displayed in HZ on the upper trace.

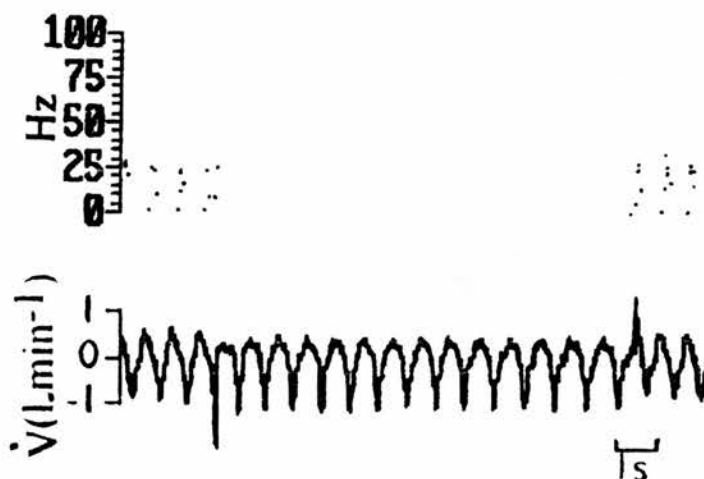


Figure 6-4: *Response of a typical slowly adapting receptor Type 1 from a control to a deflation pressure of 5 cm of water.*

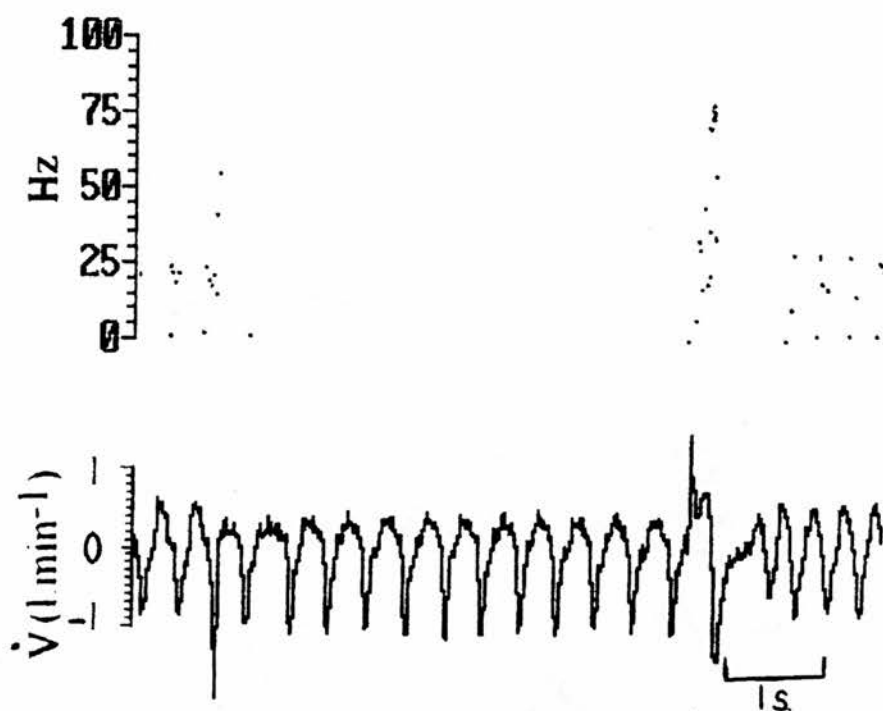


Figure 6-5: Response of a typical Slowly adapting receptor Type 1 from a control rat to a deflation pressure of 10cm of water.

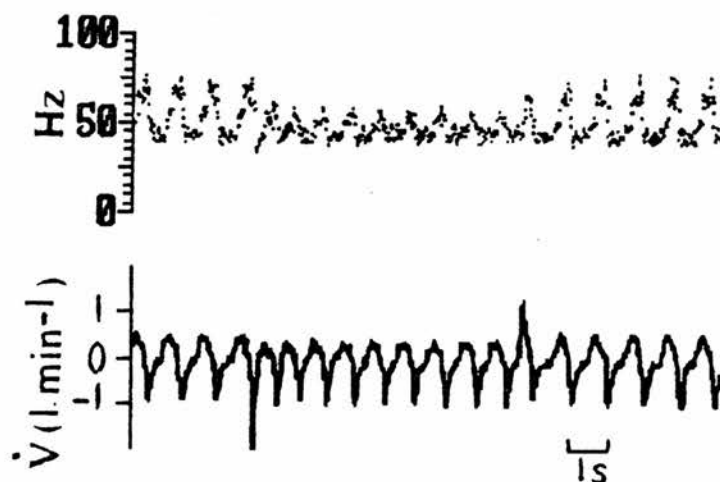


Figure 6-6: Response of a typical slowly adapting receptor Type 2 from a control rat to a deflation pressure of 5cm of water.

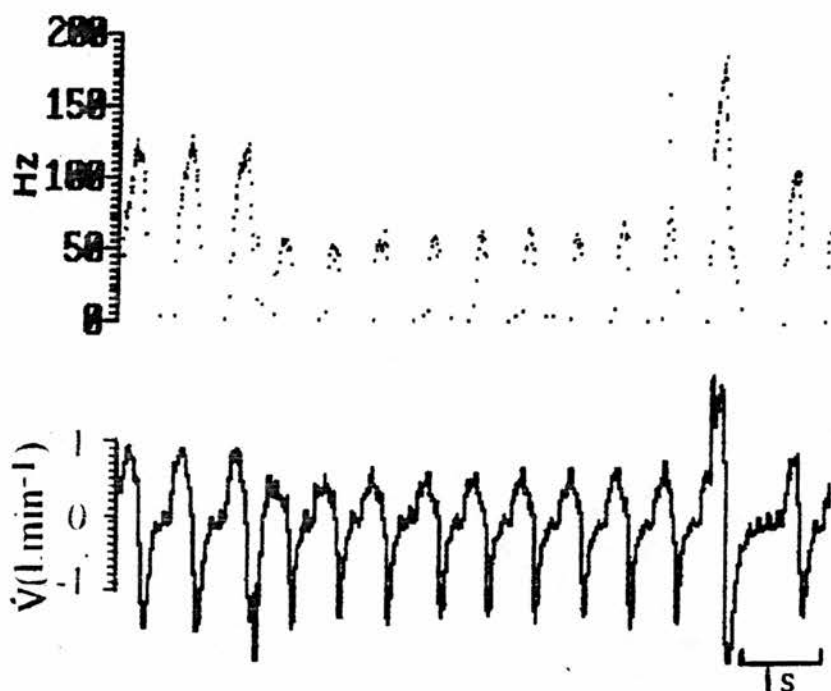


Figure 6-7: Response of a typical slowly adapting receptor Type 2 from a control rat to a deflation pressure of 10cm of water.

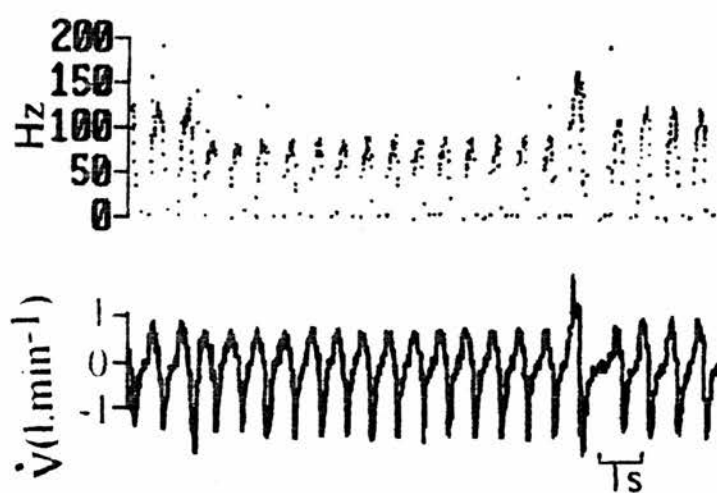


Figure 6-8: Response of a typical slowly adapting receptor Type 3 from a control rat to a deflation pressure of 5cm of water.

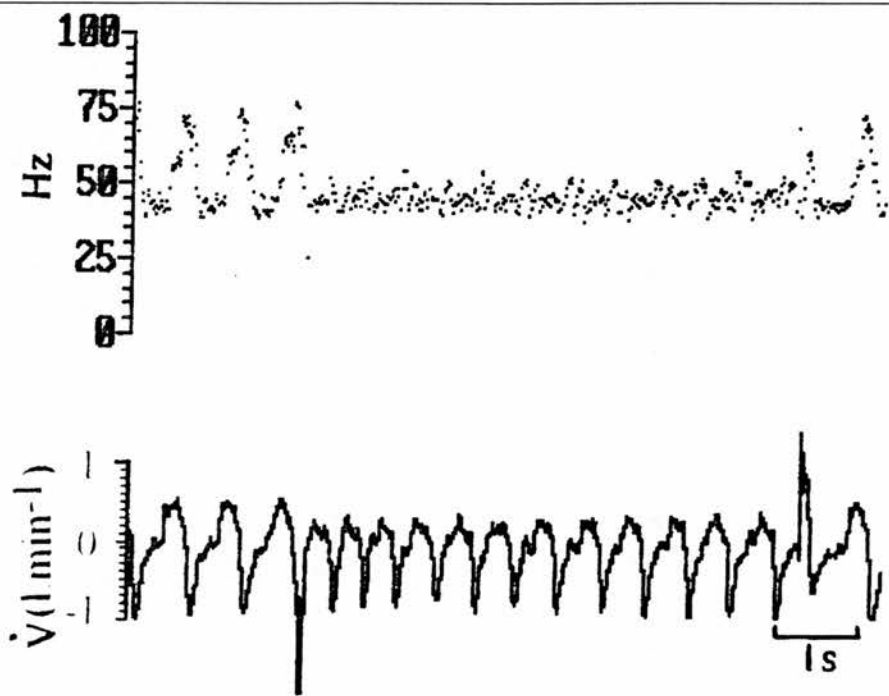


Figure 6-9: Response of a typical Slowly adapting receptor Type 3 from a control rat to a deflation pressure of 10cm of water.

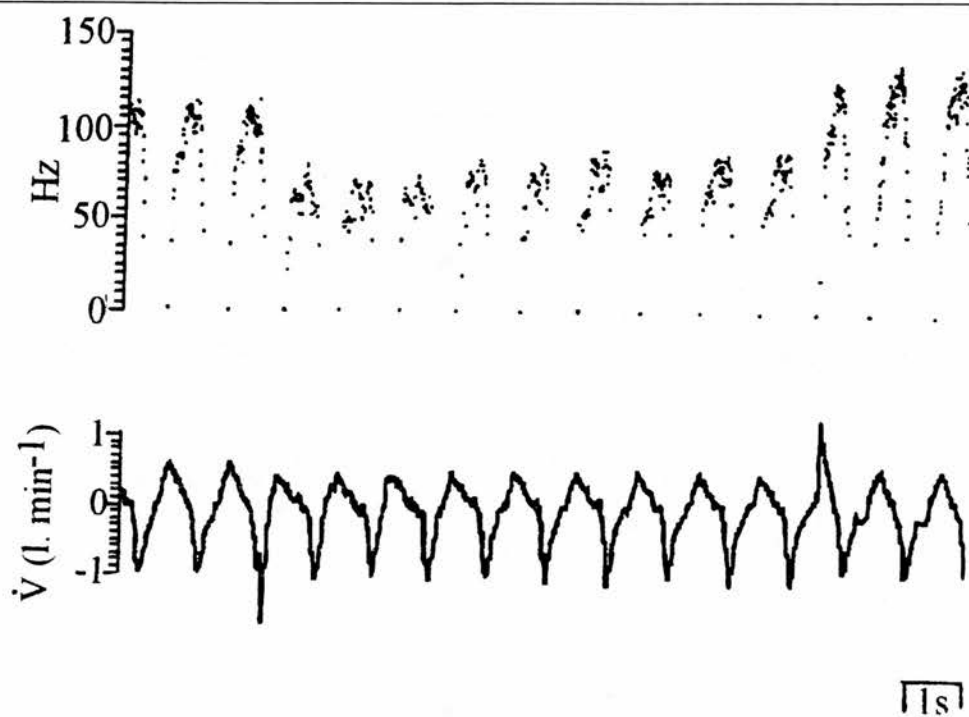


Figure 6-10: *Response of a typical Type 2 Slowly adapting receptor from an emphysematous rat to a deflation pressure of 5cm of water.*

CHAPTER 7

ACTIVITY OF PULMONARY LUNG RECEPTORS WITH CARBON DIOXIDE.

Breathing frequency did not change significantly in either the control or the emphysematous rats when carbon dioxide was administered in the unilateral vagotomised state.

	Control Rats			Emphysematous Rats		
	n=135	n=95	n=95	n=200	n=190	n=190
	Eup	4% CO ₂	6% CO ₂	Eup	4% CO ₂	6% CO ₂
Ti	0.38 +/- 0.01	0.35 +/- 0.00	0.34 +/- 0.00	0.36 +/- 0.01	0.36 +/- 0.05	0.34 +/- 0.01
Te	0.47 +/- 0.01	0.50 +/- 0.01	0.49 +/- 0.01	0.45 +/- 0.01	0.48 +/- 0.01	0.47 +/- 0.01

Table 7-1: Duration of Ti and Te in seconds for control and emphysematous rats during eupnoeic unilaterally vagotomised breathing and whilst inhaling 4 and 6% carbon dioxide in the unilaterally vagotomised state.

SLOWLY ADAPTING STRETCH RECEPTORS.

The activity of the stretch receptors while breathing carbon dioxide was similar to their activity in the eupnoeic state. The results are shown in Table 7-2. The effect of carbon dioxide on the activity of pulmonary receptors was not tested on every receptor that was recorded. The activity in 19 SARs and 13 RARs was recorded in the control rats during CO₂ administration. In the emphysematous rats, 38 SARs and 13 RARs were recorded during CO₂ administration.

ACTIVITY OF STRETCH RECEPTORS DURING INSPIRATION.

The peak firing frequency of the receptors was slightly increased in both the control and emphysematous rats when the animals were breathing carbon dioxide compared to the eupnoeic state although this was not statistically significant. The peak frequency of the control SARs in the eupnoeic state, was 86.1 +/- 1.98 HZ. During CO₂ breathing the peak frequency of the control SARs was 89.8 +/- 2.66 HZ at 4% CO₂ and 93.5 +/- 2.30 HZ at 6% CO₂. The SARs of the emphysematous rats had a peak frequency of 91.2 +/- 1.98 HZ during eupnoeic breathing and frequencies of 96.84 +/- 1.91 HZ and 96.66 +/- 1.89 HZ during 4 and 6% CO₂ administration respectively. The average number of action potentials per second was increased in the controls from 57.4 +/- 1.69 during eupnoea to 67.36 +/- 2.98 at 4% CO₂ and 68.32 +/- 2.91 at 6% CO₂. These increases were statistically significant at both levels of CO₂ (P<0.05). In the emphysematous rats the average number of action potentials per second was increased from 58.30 +/- 1.33 during eupnoea to 62.0 +/- 1.68 at 4% CO₂ and to 65.81 +/- 1.91 at 6% CO₂. These increases were statistically significant at both levels of CO₂ (P<0.05). The mean number of action potentials occurring per phase and the mean minimum firing frequency of the stretch receptors during inspiration were not significantly different in either groups of rats when CO₂ was administered.

During Ti	Control Rats 4% CO ₂	Control Rats 6% CO ₂	Diseased Rats 4% CO ₂	Diseased Rats 6% CO ₂
	n=95	n=95	n=190	n=190
Mean Peak Frequency HZ	89.8+/- 2.7	93.5+/-2.3	96.8+/-1.9	96.7+/-1.9
Mean Min. Frequency HZ	40.8+/-1.2	41.8+/-1.5	40.3+/-1.0	42.6+/-0.9
No. Action Potentials/s	67.4+/-3.0*	68.3+/-2.9*	62.0+/-1.7*	65.8+/-1.9*
No.Action Potentials/Phase	22.7+/-1.0	22.9+/-0.9	20.9+/-0.7	22.2+/-0.8

Table 7-2: Activity of pulmonary SARs of control and emphysematous rats during inspiration while breathing 4 and 6% carbon dioxide.

* represents a statistically significant difference in activity of the receptors compared to that in the eupnoeic state (P<0.05).

ACTIVITY OF STRETCH RECEPTORS DURING EXPIRATION.

During the expiratory phase when the rats were breathing CO₂ the peak frequency of the stretch receptors was not significantly different from the eupnoeic state activity. The number of action potentials per second, the number of action potentials per phase and the minimum firing frequency were also unchanged by CO₂ administration.

During Te	Control Rats 4% CO ₂	Control Rats 6% CO ₂	Diseased Rats 4% CO ₂	Diseased Rats 6% CO ₂
	n=95	n=95	n=190	n=190
Mean Peak Frequency HZ	54.29+/-3.8	57.58+/-3.4	63.36+/-2.6	65.68+/-2.4
Mean Min. Frequency HZ	22.2+/-2.2	26.44+/-2.0	26.22+/-1.7	29.77+/-1.7
No. Action Potentials/s	9.61+/-1.0	10.57+/-1.1	12.89+/-0.8	12.66+/-0.8
No. Action Potentials/Phase	4.44+/-0.4	4.92+/-0.5	6.00+/-0.4	5.88+/-0.4

Table 7-3: *Activity of pulmonary SARs of control and emphysematous rats during expiration while breathing 4 and 6% carbon dioxide.*

RAPIDLY ADAPTING RECEPTORS.

ACTIVITY OF RAPIDLY ADAPTING RECEPTORS DURING INSPIRATION.

During the inspiratory phase of breathing, when rapidly adapting receptor activity is generally low, the administration of 4 and 6% CO₂ did not change the RAR activity in either group of rats compared to the activity during eupnoeic inspiration.

During Ti	Control Rats 4% CO ₂	Control Rats 6% CO ₂	Diseased Rats 4% CO ₂	Diseased Rats 6% CO ₂
	n=65	n=65	n=65	n=65
Mean Peak Frequency HZ	15.88+/-2.66	26.59+/-3.44	23.29+/-5.08	28.8+/-5.96
Mean Min. Frequency HZ	14.15+/-4.12	11.46+/-1.21	7.21+/-1.23	8.63+/-1.51
No. Action Potentials/s	5.08+/-0.59	9.13+/-1.07	4.60+/-0.84	6.69+/-1.62
No.Action Potentials/Phase	1.74+/-0.59	3.25+/-0.39	1.6+/-0.29	2.20+/-0.51

Table 7-4: *Activity of pulmonary Rapidly adapting receptors during inspiration while the control and emphysematous rats were breathing 4 and 6% carbon dioxide.*

ACTIVITY OF RAPIDLY ADAPTING RECEPTORS DURING EXPIRATION.

During expiration the peak and the minimum firing frequency of the receptors was reduced in both the control and emphysematous rats when 4 and 6% CO₂ was given to the animals. In both groups the numbers of action potentials occurring per second were also reduced with CO₂ administration as was the number of spikes firing per expiratory phase as shown in Table 7-5.

During Te	Control Rats 4% CO ₂	Control Rats 6% CO ₂	Diseased Rats 4% CO ₂	Diseased Rats 6% CO ₂
	n=65	n=65	n=65	n=65
Mean Peak Frequency HZ	55.70+/-4.6*	56.37+/-3.7*	99.29+/-5.0	98.38+/-5.5
Mean Min. Frequency HZ	14.82+/-1.2	15.28+/-1.3	14.35+/-1.4*	12.09+/-0.9*
No. Action Potentials/s	19.45+/-1.5*	19.68+/-1.4*	27.34+/-1.5*	24.54+/-1.3*
No.Action Potentials/Phase	8.21+/-0.6*	8.62+/-0.6*	11.98+/-0.8	11.09+/-0.6*

Table 7-5: *Activity of pulmonary Rapidly adapting receptors during expiration while the control and emphysematous rats were breathing 4 and 6% carbon dioxide.*

* represents a statistically significant difference in activity of the receptors compared to that in the eupnoeic state ($P<0.05$).

CHAPTER 8.

DISCUSSION.

The purpose of this study was to investigate the pattern of breathing and vagal lung receptor activity in an animal model of emphysema. Then to relate any changes in the breathing pattern of the emphysematous rats to changes in their vagal lung receptor activity. The investigation revealed some interesting changes in the lung reflex responses and vagal lung receptor activity in the emphysematous rats compared to the controls. The pattern of breathing during eupnoea was similar in the control and emphysematous rats although the diseased rats tended to breathe slightly slower. The Hering-Breuer inflation reflex was significantly stronger in the emphysematous rats than in the controls at both 5 and 10cm of water pressure. The more severe the disease was in terms of lung compliance the longer the Hering Breuer ratio tended to be. The deflation reflex however was similar in both groups of rats. In both groups of rats breathing 4 and 6% CO₂ had no significant effect on breathing frequency. However tidal volume increased in both groups of rats when breathing CO₂, although the response was more vigorous in the controls.

There was a 2:1 ratio of SARs to RARs in both groups of rats. The SARs from both disease and control rats could be divided into three types depending on the position at which they fired during the breathing cycle.

In eupnoeic breathing both SAR and RAR activity was greater in the emphysematous rats. Within the diseased group of rats there was also a relationship between severity of the disease and eupnoeic maximum firing frequency. The more severely diseased rats tended to have SARs with higher maximum firing frequencies than those of the less severely diseased rats. The increased SAR activity could possibly explain the slight slowing of breathing in the diseased rats.

During lung inflation the SAR activity adapted significantly less in the emphysematous rats than in the controls this could be the factor responsible for the significantly increased length of the Hering-Breuer pause in the emphysematous rats. Also within the emphysematous group of rats the more diseased the rat was the less the SARs tended to adapt.

The RARs of the diseased rats had a slightly greater activity during lung deflation than did those of the controls however no significant differences were detected in the deflation reflex of the emphysematous rats compared to the controls.

Since vagal lung receptor activity was enhanced in the emphysematous rats it can be postulated that perhaps similar changes in lung receptor activity in humans

could be responsible for the altered pattern of breathing and sensation of breathlessness that occurs in emphysematous patients.

A. SELECTION OF A MODEL FOR THE PRESENT STUDY.

The advantages of using papain to induce emphysema are that it is effective, very cheap, it has been successfully used in numerous previous studies in dogs, (Marco, Meranze, Yoshida and Kimbel, 1972), rabbits, (Caldwell, 1971), hamsters, (Goldring, Greenburg and Ratner, 1968) and rats (Palecek, Palecekova and Aviado, 1967). The disadvantage of using papain in an animal model is in terms of how papain produces the emphysema, since papain is a non endogenous substance the method of emphysema production is probably very unlike the processes occurring in human emphysema. The time course of this experimental emphysema, which occurs within hours, compared to decades in human emphysema is also very different. This is a common short-coming of all the animal models of emphysema.

The present study was not concerned with replicating human emphysema exactly, rather it involved studying how changes in lung architecture produced by papain administration affect pattern of breathing and lung receptor activity in this specific animal model of emphysema.

Emphysema produced by papain is of the panacinar type, (Snider, Lucey and Stone, 1986). The commonest form of emphysema in humans is the centrilobular type. The other simple method of producing emphysema, using elastase, is more expensive than papain and the lesions produced are also of the panacinar type. Nitrogen dioxide-induced emphysema produces a type of disease which resembles centriacinar emphysema, (Snider, Lucey and Stone, 1986) however the procedure necessary to induce emphysema by NO₂ exposure is difficult, (Snider, Lucey and Stone, 1986) it was therefore decided to use papain. Elastase is a more potent producer of emphysematous lesions than papain, (Snider, Hayes, Franzblau, Kagan, Stone and Korthy, 1974) but using a larger dose of papain compared to the dose required of elastase compensates for this difference in potency and did not represent any problems in the present study. The emphysema induced with cadmium chloride together with beta-aminopropionitrile, (Cantor and Turino, 1991) is brought about by endogenous proteases and therefore perhaps mimics the actual disease more closely than the papain method, but again the emphysema is produced rapidly and is therefore unlike the natural disease; also with high death rates among animals it did not offer any particular advantage to the study over papain. With the genetic models it would have been difficult to have good animal controls as the animals would be of different

genetic strains; also these models are in mice which would present the difficult problems of recording from single fibres of the vagus in such a small species. The starvation-induced emphysema model would not be suitable for this study as the animal would be in general ill-health and under severe physiological stress.

Animal models of human lung disease are always open to criticism on how well they mimic the human condition, however this should not necessarily distract from their usefulness to answer specific questions. In this study the animal model was used to investigate how pattern of breathing, lung reflexes and receptor activity is affected by the morphological changes in the lung caused by administration of the elastolytic enzyme papain, and does not assume that this is exactly similar to the human disease situation, nor is this the most critical consideration in the choice of a suitable model as; "the usefulness of an experimental model should be judged on how well it answers the specific questions it is being used to answer, rather than how well it mimics human disease", (Snider, Lucey and Stone, 1986).

VALIDITY OF USING THE SUBSTANCE PAPAIN TO INDUCE EMPHYSEMA.

The acute injury used to produce a model of emphysema might not produce the same kind of receptor dysfunction as lung disease of longer standing regardless of the proteolytic enzyme used (papain or elastase would have the same shortcoming). An experimental model of emphysema produced by methods more closely related to the etiology of the human disease could be better in this respect.

SELECTION OF METHOD FOR PAPAIN ADMINISTRATION IN THE RATS.

USE OF AEROSOL OR INSUFFLATION.

Two methods of administration of papain were considered. The endotracheal method where papain is instilled directly into the lungs through an endotracheal cannula and the aerosol method where aerosolised papain solution is inhaled by the animal. Both methods of administration have been used to produce emphysematous lesions in other studies (Palecek, Palecekova and Aviado, 1967), (Giles, Finkel and Leeds, 1970). The endotracheal method did not involve specialised equipment and a known dose could be given.

The aerosol route of administration of elastolytic enzymes produces more uniform lesions, (Goldring, Greenburg and Ratner, 1968). A problem with the aerosol technique is that it is difficult to have any idea of how much of the aerosol gets into

the lungs. Also the aerosol method of administration would require an aerosol chamber suitable to prevent any particles of the proteolytic enzyme being accidentally inhaled by humans. This would have been expensive and was unnecessary since the endotracheal method of induction, which we tried first, proved successful and minimised potential exposure of humans to the papain.

SELECTION OF TYPE OF PAPAIN USED.

A crude preparation of papain was used in this study, rather than a purified preparation which may have elastolytic activity removed during the purification process. Crude amorphous papain preparations are more potent inducers of emphysema than papain in its purified crystalline form, (Snider, Hayes, Franzblau, Kagan, Stone and Korthy, 1974).

QUANTIFICATION OF EMPHYSEMA MODEL.

From histological investigations it has been shown that the destructive phase of the emphysematous lesion is complete by the eighth day after treatment with papain. Rats show this maximal destruction of the alveoli within eight days, (Kilburn, 1976). Since the most important demonstration of the presence of emphysema in an animal model is anatomical change, (Snider, Lucey and Stone, 1986), histological lung sections were taken from control rats and rats four weeks after treatment and assessed for significant anatomical changes.

Mean linear intercept (L_m) measurements of the alveoli of the lungs from the two groups of rats were obtained by the method of Dunhill, (1962) and the mean values for L_m compared using the Student's t-test. The papain treated rats showed statistically significant increase in L_m , ($109.3 \pm 2.7\mu m$, $n=31$) compared to the control value of ($81.5 \pm 3.1\mu m$, $n=7$) indicating that significant anatomical destruction of the walls of the alveoli ($P<0.01$) had occurred in the treated group due to administration of papain.

The L_m values obtained from the two groups of rats in this study were comparable to those cited in Eidelman, Bellofiore, Chiche, Cosio and Martin's (1990) study investigating morphometric indices in enzymatic induced emphysema. Values of L_m from their control rats were $60\mu m$ and those of their elastase treated rats were $80\mu m$. This study reported L_m values in low dose elastase treated rats that were 19.8% greater than controls and L_m values in high dose rats that were 33% greater

than controls. The rats in this work treated with a dose of 120 mg/Kg of papain had a 22% greater L_m value than the control rats.

In an investigation into the effects of different enzymes on the structure and function of in vitro rat lungs, (Johanson and Pierce, 1972) the in vitro control lungs had L_m values of 75 μ m while the papain treated in vitro lungs had L_m values of 94 μ m, a statistically significant difference. These results are similar to those from the in vivo lungs of the rats in this study.

On examining the histological lung sections from the treated rats in this study it was clear that destruction of lung tissue had occurred. The destruction was not localised. Airspace enlargement was evident in each of the fields examined on the sections which were from a large 1cm by 1cm area of the left lobe. The development of airspace enlargement, resembling human panlobular emphysema, is the pathologic hallmark of lung destruction induced by elastolytic administration to rodents, (Kaplan, Kuhn and Pierce, 1973).

Physiological measurements of increased compliance were considered as a useful secondary diagnostic indicator for the presence of emphysema and therefore lung compliance measurements were made in the two groups of rats.

LUNG MECHANICS.

It has been reported that induction of emphysema in rats does not produce changes in their airways resistance, (Boyd, Fisher and Jaeger, 1980), and so I concentrated on measurement of compliance in my model.

LUNG COMPLIANCE.

The static compliance of the control rats and emphysematous rats was measured in situ. The Cst of the control rats of 0.41 \pm 0.03 ml/cm H₂O was similar to the mean value of rat lung compliance of 0.39 ml/cm H₂O found by other workers, (Crosfill and Widdicombe, 1961), this suggests that the technique was adequate. The Cst measured from the volume/pressure inflation curve of the emphysematous rats in this study was statistically significantly greater than the control rats at $P < 0.02$. The mean Cst of the lungs from the emphysematous rats in situ measured from the slope of the volume/pressure deflation curve was statistically significantly different from the control rats, ($P < 0.01$). The slope of the volume/pressure deflation curves for both the control and emphysematous rats was significantly steeper than the inflation curve due to hysteresis of the air filled lungs. This gives a higher compliance value from the deflation rather than the inflation volume / pressure curve.

Other studies on the enzymatic induction of emphysema in rats report increases in Cst as dosage of enzyme increases, (Eidelman, Bellofiore, Ciche, Cosio and Martin, 1990). The Cst they report of 1.022 ± 0.07 ml/cm H₂O for their high dose elastase group of rats was statistically significantly different from their control group. All their values of Cst were somewhat higher than the results in this study. This could have been due to young rats, 8-10 weeks old weighing only approximately 271g, being used in comparison to the rats from this study which were much older and weighed 477-663g.

BLOOD GASES.

There was no significant differences between the blood gas values in the emphysematous and control rats. However the partial pressure of oxygen was higher in the controls than it was in the emphysematous group and the partial pressure of carbon dioxide was lower in the control rats than in the emphysematous. These results are difficult to compare with human beings as in the human disease there is usually a mixture of bronchitis and emphysema rather than pure emphysema as in the animal model.

The partial pressure of oxygen in the blood of both the control and the diseased rats were within the normal range. However since the control rats in the study had PCO₂ within normal range and the diseased rats had PCO₂ out with the normal range, this indicates that diseased rats may have suffered the similar ventilatory deficiencies as human patients. However the mechanism of these deficiencies was not pursued in this study.

From the results of increased mean linear intercept values in the treated rats, increased static lung compliance and slightly lower PO₂ and slightly higher PCO₂, it was concluded that an animal model of emphysema had been produced that could be use in the investigations into how papain-induced emphysema affects breathing pattern and lung receptor activity in the rat.

B. PATTERN OF BREATHING.

PATTERN OF BREATHING IN THE INTACT STATE.

In the present study the anaesthetised breathing frequency, before tracheal cannulation, was similar in the control and the diseased rats. After tracheal cannulation the breathing frequency fell in both groups of rats, probably due to the removal of upper airways resistance. The removal of nasal resistance in the rats by the insertion of a tracheal cannula would markedly reduce total airways resistance as nasal resistance is the largest single component of resistance in the respiratory system being one-half the total respiratory resistance and two thirds of the airways resistance, (Ferris, Mead and Opie, 1964). When the pneumotachograph was connected to the animal via the tracheal cannula the breathing frequency was slightly higher than the frequency before cannulation. This was probably due to an increase in dead space produced by the pneumotachograph. This increase in dead space was limited as far as possible by using the minimum amount of tubing and a small pneumotachograph. With the simple observational method of estimating the breathing frequency by counting, the diseased rats had a higher rate of breathing than the controls when the pneumotachograph was connected, although this was not statistically significant as the standard error of the mean was high. Measurement of breathing frequency by this method was not as precise since rats have very high breathing frequencies which makes counting liable to inaccuracies. The first time in these experiments that breathing could be instrumentally compared was when the eupnoeic breathing was recorded on tape. Later computer analysis of breath length showed that the emphysematous rats had a slower breathing frequency than the controls. Both T_i and T_e were slightly greater in the emphysematous rats compared to the controls.

The difference between the results of this work and the studies on emphysematous humans, (Loveridge, West, Anthonisen and Kryger, 1984) and horses, (Gillespie, Tyler and Eberly, 1966) could be in part due to the difference in conscious state of the animals in the different studies. In this study the animals were anaesthetised, whereas studies into breathing pattern of emphysematous patients and the COPD horses were carried out with the subjects conscious. When compared to other studies on unconscious anaesthetised animals such as Delpierre's work on anaesthetised rabbits, (Delpierre, Fornaris and Payan, 1985) the results of this study are similar, showing a slowing in breathing frequency in the emphysematous animals compared to the controls due to an increase in both T_i and T_e . No data was available on the breathing pattern of emphysematous patients in the anaesthetised state. It

appears from the differences between studies that conscious control of breathing may be of importance in controlling the pattern of breathing in emphysema. Without conscious control, emphysema may in fact produce a slowing of breathing, which is masked by the effects of conscious sensation. It can be hypothesised that this slow breathing pattern would be distressful and hence conscious animals may increase their breathing frequency to minimise dyspnoea. Restraining breathing augments dyspnoea at a constant level of PCO_2 , (Chonan, Mulholland, Cherniack and Altose, 1987). Rapid shallow breathing may be adopted by some patients with COPD as a strategy to minimise the discomfort of dyspnoea, as this sensation develops much less rapidly when breathing frequency rises, (Cherniack and Altose, 1987). Studies into the conscious pattern of breathing in rats with experimental emphysema could be easily done and compared to the anaesthetised pattern that was found during this investigation.

During eupnoeic breathing sensations of breathlessness are at their minimum, it is on exertion or exercise that dyspnoea is usually sensed. It would therefore be expected that any differences in breathing pattern or receptor activity would also be minimal during quiet breathing. The results of this study suggest that in the anaesthetised state alterations in the rats breathing patterns are minimal.

Breathing patterns in rats, like those of humans, are very variable in eupnoea from subject to subject. Therefore statistically significant differences between the control and diseased groups would be unlikely. A better control for studying changes in resting breathing pattern would be to measure the breathing pattern in the same animal before and after papain administration. However this was not possible in this study as it would have involved a series of traumatic, stages unsuitable to be all performed in the one animal, and it would have reduced the efficacy of an animal acting as its own control. The rats would have had to be anaesthetised for their control breathing pattern to be measured via a tracheal cannula and then allowed to recover. The rats would have had to undergo papain administered under halothane anaesthesia, and be left for several weeks before their emphysematous breathing pattern could be recorded again under anaesthesia. This series of procedures would have been far too traumatic for the one rat. Studies on breathing pattern under reflex conditions when changes in pattern would be exaggerated were therefore used to detect any changes in breathing pattern.

THE EFFECT OF THE REMOVAL OF VAGAL INFLUENCE ON PATTERN OF BREATHING IN CONTROL AND EMPHYSEMATOUS RATS.

In the unilaterally vagotomised state, intermediate between the intact and bilaterally vagotomised positions, both groups of rats had a slower breathing frequency than in the intact state, and a faster frequency than when both vagi were cut. The slowing of breathing observed after unilateral vagotomy is due to the partial removal of the vagal component. If a part of the stretch receptor influence is removed, by cutting one of the vagi, inspiration is not switched off as quickly and is thereby prolonged. This accounts for the increased T_i in the unilaterally vagotomised rats. The prolonged expiration can be accounted for in part by the removal of the influence of rapidly adapting receptors which trigger inspiration, (Davies, Sant' Ambrogio and Sant' Ambrogio, 1981). If the rapidly adapting receptor influence is reduced, inspiration is not triggered as quickly and therefore expiration continues for longer, resulting in the increased T_e observed in the unilaterally vagotomised rats. SAR activity also plays a part in determining expiratory time, (Davies and Roumy, 1986), the removal of the influence from SARs would tend to shorten T_e . In the unilaterally vagotomised state, when there is a reduced influence of vagal receptors, the emphysematous rats had a higher frequency of breathing than the controls. In the bilaterally vagotomised state all vagal influence was removed. The effects of the removal of the SARs switching off inspiration and the removal of the RARs, either shortening expiration or initiating inspiration, produced the slow breathing of the bilaterally vagotomised animals. The breathing frequency was slower after bilateral vagotomy, than after unilateral vagotomy as there was total removal of the excitatory vagal input to the respiratory centre in the former compared to only partial removal in the latter state. The determinants of breathing pattern are solely through chemoreceptors and the respiratory centre itself in the bilateral vagotomised state. In the absence of vagal influence the drive to breathe was greater in the diseased animals than in the controls, as would be expected from the reduced respiratory ventilation caused by loss of alveolar surface. However in the presence of vagal receptor activity, breathing is slowed more in the diseased rats and minute ventilation is decreased, perhaps illustrating the separation of drive to breathe from pattern generating mechanism.

To summarise, in anaesthetised animals it is unlikely that any higher brain centres are involved in controlling respiration, so in the vagotomised rats in this study the only remaining component that controls breathing is the chemical drive. In the vagotomised state, with no lung receptor information coming to the brain, the

diseased rats tend to breathe faster than the controls possibly due to increased chemical drive to breathe. This increased chemical drive would tend to cause the diseased rats to breathe faster than the control rats. However when the vagi are intact some component carried in the vagus nerves tends to cause the emphysematous rats to breathe slower than the controls. As the slowing effect of the vagus is greater in the emphysematous rats than in the controls this would suggest that there is a greater effect of SARs in the emphysematous rats than in the controls. Although these results were not statistically significant they perhaps give an indication of an inappropriate drive to breathe in the diseased rats.

VARIABILITY IN BREATHING PATTERN.

Since it had been reported that COPD patients have significantly fewer sighs than normal subjects, (Loveridge, West, Anthonisen and Kryger, 1984) it was thought that it would be interesting to investigate the number of augmented breaths or sighs taken by emphysematous rats compared to normal rats, to assess if this difference might be of significance in modelling the human disease. The breathing pattern during a two minute period of eupnoeic breathing, with the vagi intact, was analysed for the number of augmented breaths spontaneously occurring, as described in the Methods chapter. The result that the emphysematous rats took fewer augmented breaths supports the finding in humans. Large breaths are more expensive in terms of energy expenditure in COPD patients, because of the increased work of breathing on both inspiration and expiration in this group, (Loveridge, West, Anthonisen and Kryger, 1984). Taking big breaths may be too costly and therefore COPD patients do not sigh as frequently or take breaths of the same amplitude as normal subjects; or perhaps conscious COPD subjects may keep sigh taking to a minimum, to prevent the feeling of distress since slow deep breathing brings on dyspnoea in these patients. As hyperventilation and rapid, shallow breathing are often independent of hypoxaemia they may well be related to altered activity of neural receptors in the lungs, (Anthonisen and Cherniack, 1981). Some patients breathe more rapidly than is required merely to minimise respiratory work and are uncomfortable when made to breathe more slowly, (Paul, Eldridge and Fiene, 1960).

REFLEX RESPONSES TO INFLATION PRESSURE IN THE INTACT STATE.

In emphysema the physical environment of the lung receptors is altered. This may have an effect on the activity of certain types of pulmonary receptors. Reflex activity was studied to give a functional indication of the activity of particular groups of receptors. The Hering-Breuer inflation reflex gives a functional indication of the activity of stretch receptors in control and diseased rats in this study.

The emphysematous rats had a statistically significant increase in the strength of their Hering-Breuer inflation reflex compared to the control rats at both 5 and 10cm of water pressure. The actual apnoea produced by the lung inflation and the apnoea ratio (apnoea produced / previous eupnoeic T_e) both showed the Hering-Breuer reflex to be significantly greater in the diseased rats. This result is in agreement with a study on emphysematous rabbits which also exhibited an increased Hering-Breuer inflation reflex strength compared to control rabbits, (Delpierre, Fornaris and Payan, 1985).

As might be expected a statistically significant longer apnoea was produced when the positive 10cm of water inflation pressure was applied to the lungs in both groups of rats, compared to the apnoea produced by inflation of the lungs to 5cm of water pressure. This increased activity was probably due to the increased activity of SARs at the higher inflation pressure.

This increased strength of the Hering-Breuer inflation reflex of the emphysematous rats could be due to either increased numbers of active pulmonary stretch receptors, or an increased activity of these receptors in the diseased animals compared to normal rats.

Stretch receptor activity plateaus above a certain level of lung inflation, (Bartlett, Sant' Ambrogio and Wise, 1976), 10cm of water might maximally activate nearly all the SARs in both the diseased and in the control rats while 5cm of water might activate a smaller fraction of receptors in the controls than in the diseased animals.

Functions of the inflation reflex during physiological conditions have been postulated. It may adjust the rate and depth of breathing to be mechanically most economical, (Widdicombe, 1961) with the pulmonary stretch receptors acting as sense organs for the physical state of the lungs. If in pathological conditions such as emphysema, when the physical state of the lungs changes the normally appropriate sensing mechanism of the pulmonary stretch receptors might no longer relay appropriate information to the brain on the physical environment of the lungs.

Stretch receptors are undoubtedly a profound influence on the central pattern generator and the pattern of breathing. In human beings there has been some dispute about their role in the conscious subject; however Guz, Noble Eisele and Trenchard, (1969) demonstrated that the Hering-Breuer inflation reflex is present in anaesthetised subjects. If under any condition the Hering-Breuer inflation reflex can be demonstrated in human beings this means that the stretch receptor activity is present and just because it does not demonstrate itself in the conscious state, (which is probably because of vocalisation) except by very high threshold inflations, does not mean that the activity of these peripheral receptors is being blocked by the conscious state. To take another example of a peripheral receptor, a percinean corpuscle in the human skin will be active if the person is awake or asleep; central processing may be changed by consciousness, but never-the-less afferent information is passing up to the brain.

Although it may not operate a von Euler off switch there is no reason why stretch receptor activity should not contribute to sensation, sensation which may not be recognised in health because we adapt to activity from peripheral receptors. If this was not so we would be driven mad by our clothes for example. This activity, modified in disease, may cause the sensation of breathlessness. My thesis is that the sensation of breathlessness is from an inappropriate stretch receptor activity for the particular mechanics of the lung, and for the particular minute ventilation required to produce appropriate levels of blood gases. It must be emphasised that this activity is changed by the change in architecture of the lung which surrounds the stretch receptors. The rapidly adapting receptors on the inside of the airways will not be as profoundly altered as the stretch receptors situated in the walls of the airways on which the parenchyma pulls, and this parenchyma is altered by changes caused by emphysema. The lung is a geodesic network which means that any stress on one part of the structure is distributed evenly throughout the whole structure. If tissue is lost from the geodesic network then nodes of stress are created and these nodes of stress may cause excess stretch receptor activity in the emphysematous animals. It should be remembered that the changes in architecture produced in the emphysematous rats were quite mild compared with those seen in human emphysema.

REFLEX RESPONSES TO DEFLATION PRESSURE IN THE INTACT STATE.

I used 5 and 10cm of water negative airway pressure to deflate the lungs. These pressures were chosen as they are within the range used by other workers. I think however that 10cm of water pressure is too great a load for the rats to breathe against as breathing became quite laboured in some of the diseased rats at this pressure, as shown in Figure 8-1. A more physiological deflation pressure is 5cm of water and I will concentrate on the results at this lower pressure.

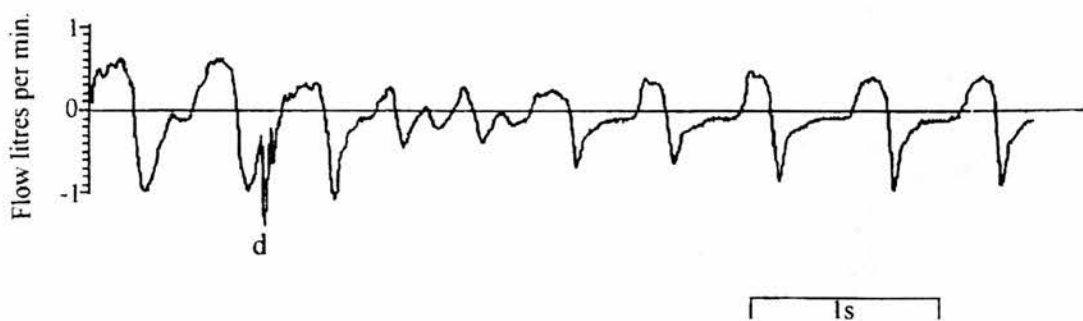


Figure 8-1: *Flow trace from emphysematous rat during lung deflation to a pressure of 10cm of water.*

Inspiration is upwards on the trace. The position at which the deflation was applied is marked by the letter d on the trace above. At the onset of deflation there were four or five very rapid shallow breaths, followed by laboured breathing as the animal tries to breathe against the negative pressure of 10cm of water.

The control rats did not appear to struggle against the negative pressure as much as the emphysematous rats. The control rats responded similarly to a deflation pressure of 10cm of water pressure as they did to a deflation pressure of 5cm of water pressure. They increased T_i to the same extent at both pressures and decreased T_e to the same extent at both pressures. However T_e by the fifth and sixth breath was slightly above eupnoeic level at the deflation pressure of 10cm of water, while at the deflation pressure of 5cm T_e was still shorter than eupnoeic level.

The results show that the diseased rats responded differently to negative 10cm of water pressure as T_e did not shorten for the first breath of the deflation to 10cm of water pressure and T_i increased in the first breath of deflation, but was below eupnoeic level by the fifth and sixth breath. This lack of shortening of T_e in the overall result for the diseased rats could be because 14% of deflation tests resulted in T_i decreasing and T_e increasing.

There seems to be no clear difference between the diseased and the control rats in the response to 5cm H_2O deflation. In 89% of deflation tests in the controls, rats responded by increasing T_i and decreasing T_e . In the diseased rats 88% of deflation tests resulted in such changes. The degree of lengthening of T_i and shortening of T_e that occurred was also similar in both groups of rats, as shown in chapter 3 Table 3-4. In deflation RARs are stimulated and SAR activity is reduced. These two things interact in complex ways with each other and with the central mechanism. The central mechanism shows refractoriness that occurs after an induced or spontaneous augmented breath, (Davies and Roumy, 1982). The fact that there are very few spontaneous augmented breaths (0.1/min) in normal rats may have something to do with this lack of effect on deflation. Although I did not see augmented breaths during lung deflation in these rats of the type taken during eupnoeic breathing, that is a biphasic inspiratory flow pattern, the first inspiration that the rats took after the deflation pressure was applied was augmented, in that the first T_i was longer than subsequent inspirations taken during the deflation.

An increase in T_i during deflation could be due to either the increased activity of RARs producing an augmented type inspiration, or a result of the reduction in SAR activity which operates an inspiratory off switch, terminating T_i .

In the diseased animals there was an increase in both SAR and RAR activity during eupnoea. The increase in SAR activity is expressed by a slowing of breathing and an increase of the Hering-Breuer inflation reflex. However I was unable to detect any expression of the increase in RAR activity. This may result from the difference in proportions of stretch and rapidly adapting receptors in rats compared to other animals, (Pirie and Davies, 1995). A small number of receptors, if their activity increases against a very low base line, may paradoxically produce a more profound effect than a large number of receptors with a high resting discharge; the prime example of this is a cough receptor, there are very few cough receptors and only one needs to be stimulated to produce paroxysmal coughing.

RESPONSE TO INHALATION OF CARBON DIOXIDE.

Increased inspired PCO_2 was used to accelerate and stimulate breathing as occurs during exercise, which is when emphysematous patients suffer the most disability due to dyspnoea.

Minute ventilation would be expected to rise with increased levels of carbon dioxide in the inspired air. An increase in minute ventilation can be achieved by an increase in respiratory frequency or by an increase in tidal volume, or a combination of the two. There was no effect of breathing air containing 4 and 6% carbon dioxide on the respiratory frequency in either group of rats. This was surprising as respiratory frequency was expected to increase with increasing levels of CO_2 as shown in rabbits, where T_e was reduced when CO_2 was added to the inspired air, (Davies and Pack, 1991). However minute ventilation did rise when the animals breathed 4 and 6% carbon dioxide as there was a statistically significant increase in tidal volume. The emphysematous rats did not increase their tidal volume as much as the control rats. Although the diseased rats had a lower mean tidal volume than the control rats at the 4% level of CO_2 , this difference was not statistically significant. When the level of CO_2 was increased to the 6% level the difference between the mean values for tidal volume became statistically significant. The reduced increase in minute ventilation of the emphysematous rats in response to breathing additional CO_2 could be due to a number of reasons. It could be that the diseased rats had a lower chemosensitive response to CO_2 . Alternatively it is known that patients with COPD breathe at higher lung volumes than do healthy subjects, (Hubmayr and Rodarte, 1991) this may mean that they cannot increase their tidal volumes as much as normal subjects. Emphysema causes a marked reduction in the lung elastic recoil, this results in an increase in functional residual capacity. Functional residual capacity and total lung capacity are increased in rats with elastase induced emphysema, (Eidelman, Bellofiore, Chiche, Cosio and Martin, 1990).

In terms of changes in tidal volume the emphysematous rats did not respond as vigorously as the control rats to inhaled carbon dioxide at either the 4 or 6% level. When the animals at rest are made to breathe a certain percentage of CO_2 it raises the partial pressure of CO_2 in the blood to a certain value and provides a chemical drive to breathe, which I assume was the same in both the control and emphysematous rats. The drive to breathe was the same but the change in tidal volume was different. This suggests that the mechanical properties of the lungs in the emphysematous rats were limiting change in tidal volume, unless there had been some blunting of the

chemoreceptors to carbon dioxide caused by retention of carbon dioxide which was not shown in the blood gases.

In humans a reduced central ventilatory response to hypercapnia is thought to contribute to CO₂ retention in some patients with COPD. It has been demonstrated that hypercapnia causes a greater diaphragmatic electrical activity in eupcapnic COPD patients than in hypercapnic patients, (Lourenco and Miranda, 1968). A pattern of breathing characterised by high respiratory frequency and small tidal volumes has been reported in some hypercapnic COPD patients. They therefore have an increased dead space ventilation compared to eupcapnic patients, (Javaheri, Blum and Kazemi, 1981). A shortened inspiratory time is believed to contribute to the reduced tidal volume observed in hypercapnic patients, (Sorli, Grassino, Lorange and Milic-Emili, 1978). Mechanisms for this altered pattern of breathing in hypercapnic patients are not known, possible explanations offered however include respiratory muscle dysfunction, stimulation of RARs in the airways and abnormalities of respiratory drive, (Wiedemann, and Matthay, 1991).

C. RECEPTORS IN EUPNOEA.

RECEPTOR PROPORTIONS

The proportion of receptors (SAR:RAR) in the 1 week and 4 week diseased rats was unchanged compared to the controls. This indicates that no loss of receptors occurred in the diseased rats due to administration of papain, or at least that stretch and rapidly adapting receptors were lost in similar proportions to each other. The result that the 1 week rats had similar proportions of receptor types to the 4 week diseased rats suggests that there was no acute or chronic loss of one particular receptor type.

It was necessary to examine the ratio of stretch to rapidly adapting receptors in the control and diseased rats, as although it is difficult to propose any mechanism whereby emphysema would increase the number of any type of the receptors it is possible that the number of rapidly adapting receptors might have been reduced by damage to the epithelial walls of the airways in the diseased rats by administration of papain. Any reduction in RAR numbers would alter the overall information travelling to the brain via the vagus nerves. A reduction in the numbers of RARs in the emphysematous rats would be indicated by an increased ratio of stretch to rapidly adapting receptors recorded from in these animals. By determining receptor proportions in the control and treated rats loss of SAR numbers could also be considered, although it would seem unlikely that loss of SARs would result from administration of papain as they are located in muscle walls of the airways. The proportion of SARs to RARs was calculated in control rats and in papain treated rats one and four weeks after papain administration, as any destruction might be a slow, or more likely, rapid process. The proportion of unmyelinated fibres was not determined in this study.

RECEPTOR TYPES AND THEIR PROPORTIONS IN THE CONTROL RATS.

In this study receptor activity was recorded in anaesthetised, spontaneously breathing rats under eupnoeic conditions and sustained lung inflations, procedures designed to identify receptors as slowly or rapidly adapting without resort to paralysis or thoracotomy, which would exclude recording under eupnoeic conditions. The difference between SARs and RARs was very clear, and did not depend on the tentative division of SARs into three types for comparison with other published

findings. The findings in this study are sufficiently similar to those previously reported for open-chested or paralysed rats to bear comparison. The recent papers, (Bergren and Peterson, 1993), (Tsubone, 1986) investigating vagal lung receptor activity have pointed out the paucity of information about the properties of pulmonary receptors in the rat and described discharge patterns mainly under conditions of respiratory paralysis and artificial ventilation.

The results of the present study differ from the results of both Bergren and Peterson and of Tsubone probably due to the differences in transmural pressure found in the open- or closed-chested rats. That opening the chest affects receptor activity is clearly demonstrated in Tsubone's paper, (Tsubone, 1986). Bergren and Peterson, (Bergren and Peterson, 1993) also show that there was a difference between inspiratory units in their open- versus closed- chested rats in terms of the number of impulses occurring per cycle. In view of the profound differences between open- and closed-chested preparations, and the fact that intrapleural pressure probably changes slightly, even over the brief interval during which adaptation was recorded, it is remarkable how similar the categories and properties of the slowly adapting receptors in Bergren and Peterson's report are to these present results. I found 21% of SARs (14% of all receptors) to be exclusively inspiratory, (Bergren and Peterson-25%). Fifty three percent of my SARs, (35% of all receptors) discharged throughout inspiration and early expiration, (Bergren and Peterson-49%). Twenty six percent of my SARs, (18% of all receptors) discharged throughout the respiratory cycle.

As spontaneously breathing rats have respiratory frequencies of the order of 100 breaths per minute, (Marshall and Metcalf, 1988) and show virtually no expiratory pause, the "deflationary (D) SARs" of Bergren and Peterson, which made up 18% of their SAR (PSR) population, and the "deflation sensitive receptors" of Tsubone, (Tsubone, 1986), both of which were stimulated during the deflationary phase of their ventilating pump, have little equivalence to any of my receptors. It is interesting to note however that Tsubone, (Tsubone, 1986) reports the rate of discharge of this deflation sensitive type of receptor was profoundly changed by opening the chest (activity changed from 21-24 to 45-53 impulses per cycle). One may speculate that these receptors would approximate more closely to one of the groups described in the present study if the rats from which they were recorded were breathing spontaneously rather than being paralysed and ventilated by positive pressure.

Table App.4-7 in Appendix 4 shows the peak and minimum firing frequencies calculated from interspike intervals, mean frequency and number of action potentials firing per phase for all the types of receptors. Mean Frequency is the number of action

potentials in a phase of breathing (inspiration or expiration) divided by the duration of that phase. This algorithm is applied to take into account variations in the duration of the phases of breathing. Figure 4-12 in chapter 4 shows the phase-spanning nature of the Type 3 receptors and that Type 1 and Rapidly Adapting Receptor discharges are highly polarised into inspiration and expiration respectively. The properties of what are called Type 1 and Type 2 receptors are very similar. They were tentatively separated into two types mainly on the basis of adaptation rates and eupnoeic frequencies of discharge. Widdicombe, (Widdicombe, 1954) comments that "adaption rate alone does not distinguish between different groups of pulmonary sense organs." It may well be that further investigation will not sustain this separation, which is not in any case central to the thesis being tested, as it only requires a discrimination between rapidly and slowly adapting receptors, clearly demonstrated in my results.

As the imposed ventilatory cycles of the paralysed rats used by other workers were so different in duration and waveform from the spontaneous breathing of the rats in this study it is difficult to make comparisons of frequency of discharge. It can be said that the total number of impulses produced in my study by Type 1 SARs, in a respiratory cycle of inspiratory duration 0.39 ± 0.005 s and expiratory duration 0.43 ± 0.013 s ($n=60$ breaths, 12 rats) is of the same order of magnitude as all except the "Mostly Inspiratory" receptors reported by Bergren and Peterson, (Bergren and Peterson, 1993) during a pump cycle of approximately 0.9s, of which approximately 0.2s was occupied by inflation. As most of the activity reported by these authors took place during inflation there is an approximation to the rate of discharge I report in the spontaneously breathing rat. Schoener and Frankel, (Schoener and Frankel, 1972), who used a realistic frequency, (2Hz) to ventilate their paralysed rats, reported a mean discharge frequency of 96 ± 7 Hz for SARs during normocapnia. This compares with the overall mean frequency for SARs in the present study.

SARs respond to the degree and rate of change of volume of the lungs, (Davis, Fowler and Lambert, 1956) and have been categorised into those that saturate above kPa transmural pressure and those with a more linear response. Some workers report that in the dog, (Pack, Ogilvie, Davies and Galante, 1986) and opossum, (Farber, Fischer and Sant' Ambrogio, 1983) there is a continuum rather than discrete SAR types. While this may be true for Type 1 and 2 SARs found in the rats of this present study, these were very different in rate of adaptation and position of firing in the respiratory cycle from Type 3 or RAR, expiratory receptors. No attempt was made to identify the location of these receptors, and some may have been extrathoracic as indicated by their activity during expiratory flow, (Sant' Ambrogio and Martola, 1977). In addition to the SARs, 30% of receptors that were active during

spontaneous breathing were rapidly adapting in my rats. This is in direct contrast to Bergren and Peterson, 1993 who found only 7% of their receptors were rapidly adapting. Tsubone (Tsubone, 1986) on the other hand, using an open chested, paralysed preparation like Bergren and Peterson's, found "irritant-like receptors" which discharged during both inflation and deflation. The only apparent difference between Bergren and Peterson's and Tsubone's methods was the use by Bergren and Peterson of a 0.3-0.5 kPa end-expiratory pressure, and the repeated use of exposures of 5-20s to dimethyl-ether vapour to silence SARs. Ether vapour is reported to stimulate RARs in guinea pigs, (Bergren and Sampson, 1982). However, concentrations of 7.5-14.5% are reported to inhibit RARs, (Widdicombe, 1954). Because of their more central and superficial position in the airways compared with SARs (Roumy and Leitner, 1980) it is likely that the RARs in Bergren and Peterson's 1993 study received higher concentrations of the vapour used repeatedly to silence SARs than the SARs themselves. I cannot say if such treatment permanently silences RARs but it may explain the difference between my results and those of Bergren and Peterson.

I maintain that RARs do exist in considerable numbers in rats and are active during spontaneous ventilation. They are present in the ratio of approximately 1 RAR to 2 SARs. This compares with ratios of 1:4 in the rabbit, (Roumy and Leitner, 1980) and 1:10 in cats, (Widdicombe, 1954). The breath durations of adults in these species is also in the ratio 2:4:10, (Widdicombe, 1961).

This supports the suggestion, (a modification of that of Bartlett and St. John's, 1979), that the respiratory frequency of a species is related to the *overall* adaptation rate of *all* of its pulmonary receptors.

RECEPTOR TYPES AND THEIR PROPORTIONS IN THE EMPHYSEMATOUS RATS.

The inspiratory active receptors of the emphysematous rats, like the controls, fell into three groups or types. This classification does not necessarily suggest that these receptors are morphologically different, as the differences observed in their pattern of activity, discharge frequencies, and adaptation rate could indicate a varying stimulus to them due to their position in the tracheobronchial tree, since the position of the receptors was not determined in this study. The receptors I termed Type 3 could be the extrapulmonary receptors referred to by Sant' Ambrogio, (1987), which are SARs active at end expiratory volume and are known as tonic or low threshold receptors. Between 27 and 60% of SARs are cited as being of this type in other species, (Sant' Ambrogio, 1987). In the SARs recorded from in this study, 28% were

of this type in the control rats and 24% in the emphysematous rats. Both terms applied to these extrapulmonary receptors have their limitations. The term "tonic" is incorrect as it implies a constant discharge rate throughout the respiratory cycle, when these receptors are actually more active during inspiration. High or low threshold implies an intrinsic difference in the receptors' level of activation, whereas the reason for their difference in activation level may depend on their environment; although the term is helpful in that it indicates where in breathing these receptors are stimulated and fire supplying information to the brain. In using the term "Types" of SARs it could be thought that I am intending to imply inherent differences in the receptors I recorded from. However this is not the case as the term merely served as useful terminology to distinguish the different firing patterns observed, and allowed analysis of the changes in the proportion of receptors displaying the different firing patterns and levels of activity during both eupnoea and inflation.

The extrapulmonary receptors are thought to be located in the larger extrapulmonary airways, where mechanical factors suggest that the same transmural pressures result in a greater stimulus to the SARs due to larger circumferential tensions as predicted by Laplace's law. The receptors classified as Types 1 and 2 may well be intrapulmonary receptors, as receptors of the intrapulmonary airways are of the higher threshold type firing mainly or exclusively in inspiration (Sant' Ambrogio, 1982). The Type 1 receptors could be located lower in the tracheobronchial tree than the Type 2 receptors since as the location of intrapulmonary SARs becomes more peripheral, an increasing number are not active at end expiration but are only recruited during inspiration, (Miserocchi and Sant' Ambrogio, 1974). An indication that these high and low threshold receptors are not intrinsically different from one another is that the fibres of these receptors have similar conduction velocities, (Sant' Ambrogio, 1982). However the conduction velocities between the SARs displaying the differing firing patterns in this study were not specifically measured and therefore I cannot comment further on this.

None of the receptors recorded from in either group of rats in this study could be classified as expiratory discharging slowly adapting receptors as described in Tsubone's study on rat vagal afferent fibres, (Tsubone, 1986). These fibres were recorded from bilaterally vagotomised, paralysed, artificially ventilated animals and the differences between these recording conditions and those of the present study have been discussed in the previous section.

Interestingly the proportion of the three types of inspiratory firing SARs were slightly different in the emphysematous rats compared to the controls. Only 7% of the inspiratory receptors of the diseased rats were classified as belonging to Type 1 (14%

controls), while 44% of them were classed as Type 2 (35% controls) and 16% classified as Type 3 (17% controls). However this did not reach statistical significance when the actual numbers of the different types of receptors present in the two groups were tested with the Chi-squared test.

Although the proportion of SARs to RARs remains the same in emphysema, within the inspiratory firing stretch receptor population there appears to have been a shift away from the high threshold Type 1 receptors in the emphysematous state. This suggests that rather than there being a change in the receptors themselves there is a change in the environment in which Type 1 receptors find themselves. Thus Type 1 receptors may find themselves in a region of low compliance in normal lungs. However in the emphysematous lung, which has high compliance, these receptors may find themselves in an environment more like that in which the Type 2 receptors are located in the normal lung. If the environment influences the pattern of discharge of the SARs and therefore which type it is placed into, then a change in the environment of a particular SAR could also change which type it would be classed as belonging to. The environment causing a receptor to display a Type 2 pattern of discharge and adaptation is therefore more widespread in the emphysematous condition, possibly due to emphysema altering the environment and the activity of the stretch receptors.

ACTIVITY OF SARs IN CONTROL AND EMPHYSEMATOUS RATS.

The stretch receptors of both groups of rats display a very regular firing pattern characteristic of this type of receptors. The number of action potentials occurring and the position at which they occurred in a breath was highly consistent from breath to breath for a particular fibre. They fired mainly during inspiration, having a statistically greater activity in the inspiratory phase compared to the expiratory phase of breathing.

The stretch receptors of the emphysematous rats fired with a statistically significantly greater peak frequency than did those of the control rats in eupnoea.

The RARs were active in spontaneous eupnoeic breathing. They fired mainly during expiration, having a significantly lower firing frequency during inspiration than during expiration with some fibres never firing in inspiration. The firing pattern of the RARs was remarkably consistent from breath to breath, both in terms of the number of action potentials occurring in a phase of breathing and the time at which these spikes occurred within that phase, (see section on RARs in eupnoea in chapter 4, Table 4-8 and Figure 4-12). This consistency however was less obvious than the regularity displayed by the SARs. The RARs had a much lower firing frequency per

second than the SARs. This lower activity of RARs can give an impression of an irregular firing pattern as there may only be a few action potentials occurring per phase of breathing. If there is an increased or decreased activity recorded from a slow firing RAR of even one or two action potentials in a breath it can produce a large percentage change in activity as there might have been a very low number of spikes previously occurring in a breath (mean number of action potentials per expiration in control rats was 13.34 ± 0.55 with a range from 2-28 action potentials per expiration). A larger number of spikes in a breath in SARs makes the pattern of activity appear more consistent in these receptors.

It has been reported that RARs have little or no spontaneous activity during eupnoeic breathing in several species; including guinea pig, (Bergren and Sampson, 1982), rabbit, (Mills, Sellick and Widdicombe, 1969) cat, (Armstrong and Luck, 1974), and dog, (Sampson and Vidruk, 1975) and therefore are not thought to be involved in the regulation of normal breathing. However these studies into RAR activity in breathing were carried out in paralysed, artificially ventilated, and in the case of guinea pigs, cats, and dogs, open-chested animals. In my study, anaesthetised, closed-chested rats were breathing spontaneously. These much more physiological conditions under which RAR activity was investigated in rats was quite different from the conditions in the other studies.

In this study during spontaneous eupnoeic breathing RARs from control rats fired 4.99 ± 0.55 action potentials per second in inspiration and 28.77 ± 1.25 action potentials per second in expiration. However in other studies where the animals are open-chested and artificially ventilated, RAR activity has been reported as being very much lower. Sampson and Vidruk, (1975) using dogs and Bergren and Sampson, 1982 using guinea pigs report average frequencies from RARs of only 0.2-0.3 action potentials per second with lung ventilation at normal resting rates and tidal volumes. The significant difference in activity of RARs between these studies and the present study is likely to be due to the different conditions under which activity was recorded. In a study investigating receptor activity under similar conditions to those in my investigation, RARs from spontaneously breathing rabbits fired 14 ± 8 impulses per breath, where breath duration was of approximately 1.1 seconds duration, (Davies and Roumy 1982). This activity was similar to that in the spontaneously breathing rats in my study.

In the emphysematous rats the rapidly adapting receptors had statistically significantly greater peak, number of spikes per second and number of spikes per phase during expiration than did the controls. Little can be discussed about activity of

these receptors during inspiration as such low number of spikes (2.59 ± 0.23 action potentials per inspiration) occur in this phase.

The changes in receptor activity in eupnoea in the emphysematous rats, particularly the increased activity of the SARs, could explain the slight slowing of breathing with an increase in duration of inspiration and expiration in these rats compared to the controls. The picture in eupnoeic breathing in these animals is confused because there is no separation between the increased activity in RARs and the increased activity in stretch receptors. It has been demonstrated that RARs can produce augmented breaths in rabbits, (Davies and Roumy, 1982) although there was not any increase in duration of inspiration unless an augmented breath is provoked. Never the less there is evidence that RARs are pro-inspiratory, (Davies, Sant' Ambrogio and Sant' Ambrogio, 1981). The increased SAR activity quite clearly explains the increased duration of expiration. This confusing picture could be resolved by using a series of differential blocks, first to eliminate SARs then to take out all other myelinated fibres, that is RARs, while analysing the phrenic activity of the animal.

D. LUNG RECEPTORS DURING INFLATION.

ACTIVITY OF SARS DURING INFLATION.

The SARs displayed the characteristic long lasting discharge in response to a maintained lung inflation. The activity of the receptors declined rapidly immediately upon inflation and slowed progressively into a sustained firing rate. These adaptation processes have been related to the viscoelastic properties of the muscular tissue containing the SARs, (Davenport, Sant' Ambrogio and Sant' Ambrogio, 1981). The SARs of the emphysematous rats adapted less than those of the controls.

Bartlett and St. John, (1979) in a study of pulmonary stretch receptors in six species ranging in size from hamsters to dogs, make the important observation that adaptation of pulmonary mechanoreceptors imbues them with dynamic as well as static response characteristics. The afferent information reaching the brain from such receptors depends on both lung volume and its rate of change. It would also seem important to consider the degree of adaptation that takes place in a time interval approximately similar to the animal's respiratory cycle. The classical adaptation index of Knowlton and Larrabee, (1946) compares receptor discharge at the beginning and end of a two second lung inflation. A rat might take 3 breaths in that time interval. Widdicombe, (1954) further points out that such an adaptation index "does not

distinguish between adaptation rates of endings which cease firing within 1 second, so that stimuli well above threshold must be used and the endings must discharge for 2 seconds or more". To overcome some of these difficulties the degree of adaptation that took place within 0.75 seconds of the application of a step stimulus was measured in this study. It has already been established, (Bergren and Peterson, 1993) that rats have SARs with adaptation indexes, measured in the conventional open-chested preparation, which are similar to those of other species. The method of measuring adaptation used in this study applies a more rigorous test of whether a receptor is rapidly adapting than do the conventional methods. Bartlett and St. John, (1979) postulated that pulmonary stretch receptors of animals with different eupnoeic breathing frequencies would be expected to have different rates of adaptation, to accurately signal lung conditions to the central respiratory complex. This did not prove to be the case from their results. However, their observations were restricted to pulmonary stretch receptors. Rapidly adapting receptors are found in the lungs of many species. It may be that a change in the proportion of these receptors, relative to the number of slowly adapting stretch receptors, provides the different overall degree of adaptation required by different species, (Widdicombe, 1961).

The association of adaptation rate with frequency of breathing suggested by Bartlett and St. John, (1979) receives support from the report by Davies and Roumy that rapidly adapting receptor activity exerts a profound effect on the duration of expiration, and hence represents a powerful mechanism for accelerating breathing, (Davies and Roumy, 1982).

Adaptation rates of the receptors were measured using an algorithm, and under conditions, which differed from the adaptation index of Knowlton and Larrabee, (1946). The number of action potentials in the third 0.25s from when the step of inflation was applied was subtracted from the number in the first 0.25s of inflation and the result expressed as a percentage of the number of action potentials in the first 0.25s. This method of expressing adaptation addressed the problems of matching the period investigated to a physiological breathing pattern, and Widdicombe's criticism, (Widdicombe, 1954) concerning the problem of attributing an adaptation index to receptors which silenced within 1s of applying inflation. This form of index also addresses a problem associated with the use of "peak frequency" in the calculation of an adaptation index. Peak frequency, by definition, measures the time interval between only two action potentials. The use of a longer, albeit very brief, interval in this study provides a more representative sample of receptor activity on inflation. This form of adaptation index enabled the three types of SAR and the RARs, which we found discharged almost exclusively in expiration, to be clearly distinguished.

When the lungs of both groups of rats were inflated to pressures of 5 and 10cm of water the SARs became excited as shown by their statistically significant increases in the number of action potentials firing per second compared to the immediate inspiration before inflation (see Table 5-1). The SARs in the airways of the emphysematous rats were stimulated slightly more than those of the control rats by the same inflation pressure. This slightly increased firing rate of the SARs of the diseased rats was not however statistically significant.

When the activities of the three types of SARs were considered separately to see if any changes occurred, the Type 2 and 3, the lower threshold receptors, displayed a slightly greater increased discharge in the first 0.25 second of inflation compared to the Type 2 and 3 receptors of the control rats, at the inflation pressure of 5cm of water. However at the inflation pressure of 10 cm of water there was very little difference in the number of action potentials fired by the different types of SARs. So the greater activity of the SARs from the emphysematous rats compared to the controls could only be seen when all the SARs were considered as a single population at the inflation pressure of +10cm of water.

SARs from both groups of rats showed characteristic slowly adapting properties to lung inflation. The SARs of the emphysematous rats had statistically lower adaptation to inflation than did those of the controls at both 5 and 10cm of water pressure, when all the types were taken together (see Table 5-2). This lower adaptation was also seen between the different types of SARs in the control and emphysematous rats; with the Type 1 SARs of the controls having a higher adaptation to inflation than the emphysematous rats, Type 1 receptors. This was the case for Types 1, 2 and 3 at inflation pressures of both 5 and 10cm of water. Since the adaptation to inflation was lower in the diseased rats the increased discharge of the SARs in inflation was maintained longer. The initially greater increased activity of the SARs in the emphysematous rats, and more so the effect of this activity being statistically significantly longer lasting, could be responsible for the increased strength of the Hering-Breuer inflation reflex (length of the Hering-Breuer pause) of the emphysematous rats. This greater activity and longer lasting (slower adapting) activity of the SARs in the lungs of the emphysematous rats would be relayed via the vagus nerve, to the respiratory neurones in the brain and could influence and change the pattern of breathing, reflex activity and perhaps, though speculatively, sensation of breathing.

The reduced adaptation index, in other words increased persistence of firing in SARs, exists in two parts. There is a slightly greater increase in the firing frequency on inflation in the emphysematous rats, and this increase in frequency persists for

longer in the diseased animals than in the controls. This may be related to the lung being a geodesic network, so that any stress in a normal lung is uniformly distributed throughout it. The emphysematous lesions produced by the papain might have changed the properties of the lung so that there is a non-uniform distribution of stress, and it is stress that stimulates SARs as shear of one region of the lung from another. It could be that these lungs are reaching the end of their elastic component at lower pressures than normal lungs. Therefore if you were to think of the lung as a spring and plunger arrangement so that when you pull on the lung, when the spring is weakened, the pull gets transmitted to the tissue around the receptor more completely and without any buffering or elastic component to take up the stress. This theory is also supported by higher peak frequency in diseased SARs and RARs in the emphysematous rats.

There was a greater difference in the adaptation index of diseased and control rats at 5cm H₂O inflation than at 10cm H₂O inflation. This may be due to differences in the stimulus response curve which are greater at the lower pressure than at the higher, where response may plateau at the peak of an S-shaped curve so commonly seen in biological systems. The differences between the receptor adaptation in diseased and control rats are greater at 5cm than at 10cm of water pressure. However the differences, although less at the greater pressure, are still statistically significant and so it is unlikely that the flat part of the S-shaped curve was reached at this pressure, but rather being on the less steep region of the curve than at the 5cm H₂O pressure situation.

The increased activity of the SARs of the emphysematous rats during eupnoea and during lung inflation could be the result of increased lung compliance. A given change in pressure would produce a greater expansion of the lung producing greater stimulation of SARs. The Hering-Breuer inflation reflex was prolonged in the emphysematous rats in this study, in emphysematous rabbits, (Delpierre, Fornaris and Payan, 1985) and in an anaesthetised patient with panacinar emphysema, (Guz, Noble, Eisele and Trenchard, 1969). These results indicate that there is an increased SAR activity in emphysema. This has been confirmed from the analysis of SAR activity in the emphysematous rats of the present study showing significantly increased peak frequency in eupnoea and increased number of action potentials firing per second during lung inflation and significantly lower adaptation to inflation. In the studies investigating the strength of the Hering-Breuer inflation reflex, the increased strength of the reflex assessed in both the emphysematous human and the emphysematous animals, was associated with increased lung compliance. Since the Hering-Breuer inflation reflex is not sensitised by pulmonary vascular disease, or in infiltrations which

make the lungs stiff, (Guz, Noble, Eisele and Trenchard, 1969) the sensitisation of the reflex was probably due to the increased lung compliance producing greater stimulation of the SARs in the walls of the airways.

In a study of lung reflex and receptor activity in a rabbit model of pulmonary fibrosis, the fibrotic rabbits had a significantly reduced Hering-Breuer inflation reflex compared to control animals, (Davies and Pack, 1991). This decreased reflex was associated with a decreased lung compliance, (Davies and Pack, 1991). However since the SAR activity of the fibrotic rabbits was increased in this study it was postulated that the reduction in the Hering-Breuer reflex was due to the input from the RARs overpowering the SAR activity, (Davies and Pack, 1991). The RAR activity was not analysed, although a significantly greater number of augmented breaths, which are attributed to RARs, were found in the fibrotic rabbits. This suggests that there was a greater influence from the RARs in the fibrotic animals than in the controls, (Davies and Pack, 1991).

It is difficult to interpret the results between studies into the different lung diseases, as the combination and relative balance of the activity from the various receptors may be different between the diseases. Perhaps in emphysema the increased activity from the SARs leads to an increased strength of the Hering-Breuer reflex and a slowing of breathing as RARs are not overpowering their effect while in lung fibrosis there is also increased SAR activity but this is overpowered by a stronger influence from the RARs causing a shortening of the inflation reflex and rapid shallow breathing.

The compliance of the lung depends on their previous volume history. The viscoelastic properties of the smooth muscle of the airways, in parallel with SARs and determining their discharge characteristics, is in part responsible for this compliance. Changes in compliance brought about by lung volume manipulations will therefore naturally effect discharge properties. For example increase in compliance by hyperinflation would reduce muscle tone and SAR discharge, (Sant' Ambrogio, Sant' Ambrogio and Fisher, 1988). However increasing lung compliance by hyperinflation is very different from the increased compliance of the emphysematous lung where there is loss of elastic recoil of the lung due to destruction of the lung parenchyma. Sant' Ambrogio, Sant' Ambrogio and Fisher's, (1988) pointed out that " although airway stretch receptor discharge is usually related to transpulmonary pressure, this relationship is not always present in the case of peripherally located endings; this is possibly due to a discrepancy between local transmural pressure and overall transpulmonary pressure." Such discrepancies would be exaggerated if the homogeneity of lung structure was reduced. The SARs in Sant' Ambrogio, Sant'

Ambrogio and Fisher (1988) study responded in a variety of ways depending on their location in the lungs. The majority of receptors decreased their discharge, others increased their activity while in other receptors activity was unchanged by the increased lung compliance due to hyperinflation. In emphysema the destruction of the lung parenchyma alters the uniformity of the lung. Some regions of the lung will have increased compliance due to loss of elastic recoil while perhaps in other regions collapse of alveoli will have occurred producing a situation where localised decreased compliance exists, with a critical opening pressure being required to reopen the alveoli. Widdicombe showed that SARs have a markedly reduced volume threshold for activation when lungs are initially reinflated from collapse, (Widdicombe, 1961). So in regions where alveoli have collapsed the SARs would be activated by at a lower volume threshold, this might account for the shift toward lower threshold receptors in the emphysematous rats in the present study.

It has been suggested that RARs are stimulated by atelectasis of the lung, (Sant' Ambrogio, 1982) any form of deformation or collapse of the airways in emphysema might cause the increase in RAR activity found in the emphysematous rats of the present study.

Uniform structures resist deformation better than non-uniform structures. It may be that the normal lung being a geodesic network resists deformation better than emphysematous lungs where the uniformity of the lung is destroyed. The airways of the lungs consist of tubes joined together at bifurcations considering the tubes as cylindrical a first approximation would suggest that these are better able to resist deformation than the bifurcations at which the RARs are concentrated and which are forci for distortion.

It has been proposed that airway collapse or deformation may stimulate airway afferents that are responsible for unpleasant respiratory sensation, (Ingram and Schilder, 1967). It is thought that the pursed-lips breathing during expiration, adopted by many COPD patients, provides a mechanism for minimising the collapse of intrapulmonary airways during expiration thereby reducing stimulation of the receptors that might cause dyspnoea.

The environment of the airway receptors will not be uniformly changed in lung disease and there will be many different mechanical factors altering their activity. In the diseased lung there will be increased airways resistance due to collapse of peripheral airways, hyperinflation due to air being trapped in the alveoli, increased lung compliance due to loss of elastic recoil and areas of alveoli collapse all of which may effect receptor activity.

ACTIVITY OF RARS DURING INFLATION.

RARs of both groups were stimulated by lung inflation. This increased activity adapted rapidly in the RARs of both groups of rats, and within 0.25s from the onset of the inflation the receptors had stopped firing completely. This is a characteristic response of RARs.

E. LUNG RECEPTORS DURING DEFLATION.

RARs of both groups of rats were stimulated by deflation. The RARs of the emphysematous rats had a greater activity during deflation than did those of the control rats, although this was not statistically significant.

Lung deflation by negative pressure diminishes stretch receptor activity, (Knowlton and Larrabee, 1946) and increases rapidly adapting receptor activity (Davies and Roumy, 1982). J receptors however are stimulated only under extreme degrees of lung collapse and even then their response is slight, (Paintal, 1973). The results from the present investigation on activity of SARs and RARs agree with these studies although unmyelinated fibres were not recorded from.

At the deflation pressure of 5cm of water peak frequency and number of action potentials occurring per phase and per second tended to be greater in the RARs of the diseased rats during the 1st, 5th and 6th breath compared to the controls (see Tables 6-1 and 6-2). This slight increase in activity of the emphysematous RARs during deflation pressure compared to the controls did not express itself as a difference in reflexes. No difference in the response to the deflation pressure of -5cm H₂O was observed between the groups. Although the diseased rats had a higher RAR activity than the control rats during lung deflation to 5cm of water pressure there was no evidence of this increased activity from the deflation reflex response, as this was similar in both groups of rats. This could have been due to any effect of the RAR activity being masked by the reduced SAR activity that occurs during deflation. The response of the SARs during deflation was not analysed in detail so there may have been differences in the SAR activity during deflation in the two groups, although this was not obvious from observing the records of such activity.

Deflation of the lungs by a pressure of -10cm H₂O produced the paradoxical situation where the number of action potentials per phase in the 1st breath was slightly greater in the emphysematous RARs but by the 5th and 6th breath the control RARs fired more per phase. The number of action potentials per second and the peak frequency tended to be greater in the controls during the deflation, although this was

not a consistent finding (see Tables 6-5 and 6-6). This supports the suggestion that deflations of -10cm H₂O are so non-physiological in rats to be of little use as an experimental tool.

The SARs of both the control and emphysematous rats markedly reduced their activity in response to deflation pressures of 5 and 10cm of water.

F. ACTIVITY OF RECEPTORS WHEN THE RATS INHALED CARBON DIOXIDE.

SLOWLY ADAPTING RECEPTORS.

SARs in both groups of rats in the present study showed an increased activity at both 4 and 6% CO₂ compared to their activity during eupnoeic breathing. This stimulation could be due to an indirect stimulation caused by the increase in tidal volume seen when the animals inhaled carbon dioxide. Alternatively the stimulation could be acting by an unknown component resulting in increased bronchomotor tone leading to increased activity of SARs, however this would seem unlikely as hypocapnia, due to occlusion of the pulmonary circulation, leads to bronchoconstriction, (Sant' Ambrogio, 1982). Direct stimulation by carbon dioxide in the concentrations used would seem unlikely as the SARs, are situated deep in the airway wall and would therefore be difficult to stimulate directly.

The use in this thesis of CO₂ to stimulate breathing might be argued to depress the activity of SARs (Davies and Roumy, 1986). However most studies show that sensitivity of SARs in the lung to physiological levels of CO₂ in the intact animal is low, (Davies and Roumy, 1986) and in the present study the SARs were in fact shown to be significantly stimulated by CO₂.

Several studies suggest that SAR activity is inhibited by carbon dioxide, (Schoener and Frankel, 1972), (Mustafa and Purves, 1972), (Bradley, Noble and Trenchard, 1976). In this paper non-physiological levels of carbon dioxide were used. Experimenters increased the airway CO₂ in their dogs on cardiopulmonary bypass from 0.3% to 8% and recorded a decrease in SAR activity from 87 to 64 Hz. This was a change of airway PCO₂ from that of room air to an almost anaesthetic level. Another criticism is that they were using inhaled CO₂ to stimulate these receptors, and to suggest that this effect produces acceleration of breathing is dubious for the reason that under normal circumstances during inhalation the CO₂ in the airways falls to almost zero and in exhalation rises to 4%. This 4% CO₂ hardly has time to diffuse

in to where the SARs are located, fairly deep in the smooth muscle of the airways, before zero carbon dioxide comes into the airways again. In the Bradley, Noble and Trenchard (1976) paper where the animals inhaled 8% CO₂ this meant that the receptors were bathed constantly in 8 or greater than 8% CO₂ probably for several minutes before the effect reached its peak. Therefore carbon dioxide level was raised to levels that would not be reached in a normal breathing animal where half the time, assuming inspiration and expiration are about equal, the airways are filled with 0% CO₂.

In such non-physiological studies inhalation of CO₂ appears to depress pulmonary slowly adapting receptor discharge. The depression of SAR activity under these conditions seems not to be dependent on PaCO₂ levels, since when PaCO₂ levels are unchanged in experimental situations where there is an absence of a functioning pulmonary circulation, inhaled CO₂ still reduces activity of SARs, (Bradley, Noble and Trenchard, 1976).

RAPIDLY ADAPTING RECEPTORS.

Rapidly adapting receptor activity in both groups of rats was reduced during inhalation of 4 and 6% CO₂ in the present study. However other studies have shown, in experiments where pulmonary artery ligation results in reduced CO₂ levels, that RAR activity increases. When this effect was reversed and CO₂ levels return to normal the RAR activity diminishes, (Coleridge, Coleridge and Banzett, 1978). In animals artificially ventilated at constant rate and volume, increases in CO₂ were reported as having little effect when CO₂ concentrations were above normocapnic levels, (Sant' Ambrogio, 1987); however in rabbits a slight increase in RAR activity was noticed, (Sellick and Widdicombe, 1969).

One explanation of the inhibition of RARs by CO₂, in the spontaneously breathing rats of the present study, may be a direct effect as RARs, unlike SARs, are located superficially in the airways and therefore can be acted upon that much quicker by carbon dioxide.

G. RELATIONSHIP BETWEEN NEUROPHYSIOLOGICAL CHANGES AND SEVERITY OF THE EMPHYSEMA.

In a limited number of rats comparisons between the severity of the disease and neurophysiological data revealed that there was a relationship between disease severity and the changes in lung reflex responses and vagal lung receptor activity. The more severe the disease was in a particular rat the longer the Hering-Breuer ratio tended to be. The firing frequency of SARs and their adaptation to inflation was also related to the disease severity. As severity of the disease increased the maximum firing frequency of the SARs also increased and the less these receptors adapted to lung inflation. These results were obtained from only four rats for which both neurophysiological and lung compliance data was available and therefore the results have to be treated with caution. However these results indicate that it is the extent of the emphysema that is producing the neurophysiological changes in the papain treated rats.

H. ORIGIN OF CHANGES IN RECEPTOR ACTIVITY IN EMPHYSEMA.

In this thesis changes in vagal lung receptor activity were shown to occur in the emphysematous rats. These rats had significantly higher lung compliance values than the controls. The emphysematous rats had an increased activity of both SARs and RARs in eupnoea, increased frequency of firing and slower adaptation of SARs during lung inflation, and a slightly increased RAR activity during deflation compared to the controls.

To elucidate the mechanism responsible for increasing the vagal receptor activity in the emphysematous rats two possible factors must be considered. Firstly is it the treatment inducing the emphysema or secondly the considerable lung volume changes occurring in emphysema that are responsible for altering the receptor activity.

When lung compliance was reduced in rabbits due to induction of lung fibrosis the SAR activity increased, (Davies and Pack, 1991). In another study into the relationship between lung compliance and SAR activity in dogs, increased lung compliance due to hyperinflation reduced the activity of SARs, (Sant' Ambrogio, Sant' Ambrogio and Fisher, 1988). From the results of such studies there appears to be an inverse relationship between lung compliance and SAR activity, i.e. when compliance increases SAR activity decreases and when compliance decreases SAR activity increases.

However in the present study there is a positive relationship between lung compliance and SAR activity. When lung compliance was increased in rats due to induction of emphysema with Papain the SAR activity increased. It is unlikely given the inverse relationship between lung compliance and SAR activity established in the other studies, (Davies and Pack, 1991) and (Sant' Ambrogio, Sant' Ambrogio and Fisher, 1988) that the enhanced lung receptor activity in this study would be due to the lung volume changes and that it was probably the structural changes produced by the method of inducing the emphysema that lead to the changes in receptor activity.

In the study into the relationship between lung compliance and SAR activity in dogs increased lung compliance reduced rather than increased the activity of SARs, (Sant' Ambrogio, Sant' Ambrogio and Fisher, 1988) this was quite different to the results of increased lung compliance in the present study where SAR activity increased as lung compliance increased. The different results in the studies is likely to be due to the method by which lung compliance was increased in the two studies. In the study on dogs lung compliance was increased by hyperinflation, when lung compliance was induced by this method reduction in SAR activity occurred while in the present study when lung compliance was increased as a result of Papain administration SAR activity increased. These reported differences are probably due to the method by which increased lung compliance was induced. When increased lung compliance is the only factor altering SAR discharge their activity decreases. However when increased lung compliance is due to destruction of lung parenchyma as is the case after Papain administration the SAR discharge increases. It can therefore be concluded that the increases in vagal lung receptor activity shown in the present study is likely to be due to the action of Papain and the resulting destruction of normal lung receptor environment rather than the resulting increased lung volumes.

Since uniform structures resist deformation better than non-uniform structures in the emphysematous lungs, where uniformity is destroyed, any deformation occurring may lead to a greater stimulus being applied to the lung receptors in the airways and therefore an increase in their activity.

I. SUMMARY

The purpose of this closing section is to summarise the results of this thesis, and to discuss the significance of the research in relation to the initial objectives and to future studies into emphysema.

The objectives were:

- a) to develop a suitable animal model of pulmonary emphysema,
- b) to investigate pattern of breathing and lung reflex activity in normal and emphysematous rats,
- c) to record from single vagal afferent fibres of normal and emphysematous rats during conditions of eupnoea, lung inflation, lung deflation and whilst breathing CO₂ rich air, and finally
- d) to account for changes in pattern of breathing and lung reflex activity in terms of changes in lung receptor activity between the two groups of animals.

Emphysema was induced successfully in rats by means of an endotracheal instillation of papain. The emphysematous rats had a slightly slower breathing rate than the controls when the vagi were intact. This slowing of breathing in eupnoea could be accounted for by the greater eupnoeic activity from the SARs of the emphysematous rats compared to those of the controls. Without the influence of the vagi, (vagi cut) the emphysematous rats breathed slightly faster than the controls, possibly due to their higher PCO₂ and lower PO₂. The emphysematous rats exhibited a prolonged Hering-Breuer inflation reflex compared to the control rats. This was accounted for by the greater firing rate and slower adaptation response of the SARs of the diseased rats on lung inflation. The response to lung deflation was similar in both groups of rats. The activity of the RARs was greater in the emphysematous rats during eupnoeic breathing than in the controls. During lung deflation when RARs were stimulated the emphysematous rats had a slightly greater activity than those of the controls, but this difference was not expressed in the deflation reflex perhaps due to masking by reduced SAR in deflation.

When the rats breathed 4% and 6% carbon dioxide in their inspired air the minute ventilation of both groups of rats increased as tidal volume was raised. Frequency of breathing did not seem to be affected. The control rats responded more vigorously to breathing carbon dioxide as they increased their tidal volume more than the emphysematous rats. This could have been due to architectural change in the lung

limiting increases in tidal volume in the diseased rats, or chemoreceptor blunting to carbon dioxide in these animals.

SAR activity increased in both normal and diseased rats in response to breathing 4 and 6% CO₂, possibly due to an indirect effect of the increased tidal volume or to an unknown component on bronchomotor tone. RAR activity decreased in both groups of rats when they were breathing both 4 and 6% carbon dioxide. This could have been a direct effect on the RARs as they are located in the epithelial walls of the airways.

It was difficult to assess the significance of the changes in the pattern of breathing between the control and emphysematous rats but it seems possible that actual differences between the groups could exist. This could be addressed by following the changes in pattern from the normal state to the emphysematous state in the same animal using conscious recordings in a whole body plethysmograph.

POSSIBLE IMPLICATIONS OF THIS THESIS FOR THE HUMAN DISEASE.

Since there were significant differences in the activity of SARs in the emphysematous rats compared to the normal rats that account for the slower breathing and enhanced inflation response of the diseased animals it is possible that altered sensory information going to the brain in emphysema could be responsible for the changes in breathing pattern and sensation of breathing that exist in the human disease. As the greater RAR activity in the emphysematous rats was not expressed by a change in reflex response or breathing pattern their effect was perhaps being masked by SAR activity, therefore further work involving the blocking of SARs is necessary to elucidate the contribution made by RARs to altered pattern of breathing. Although I could not conclude from the results of this study that the increased RAR activity altered breathing pattern in emphysema it is quite possible that this increased activity could be responsible for the sensation of dyspnoea, particularly since RARs, stimulated by PGE₂ augments dyspnoea in humans.

There may be a contradictory command in emphysema being relayed from the vagal lung receptors to the respiratory neurones of the medulla since the increase in SAR activity recorded in the emphysematous rats would tend to extend expiration while the increased RAR activity would be relaying information to the brain shortening expiration. These contradictory commands to the respiratory neurones of the brain might be responsible for the sensation of dyspnoea. Although these commands are occurring in different phases of breathing the SARs of the emphysematous rats were more active during expiration than those of the control rats

as there was a shift away from Type 1 receptors, which only discharged in inspiration. Therefore during expiration, there would be more activity signalling prolongation of expiration and more RAR activity signalling shortening expiration in the emphysematous rats leading to an inappropriate pattern of stimulation of the respiratory neurones.

It is my thesis that changed receptor activity in emphysema leads to changes in respiratory drive and the inefficient breathing patterns and dyspnoea seen in human patients with this disease.

FUTURE WORK.

This study has examined the vagal input from SARs and RARs to the respiratory centre in the medulla. The present study provides no information about the activity in unmyelinated fibres in the emphysematous lungs of rats. Attention has been drawn to their activity during lung injury, (Guz and Trenchard, 1971), however little quantitative detail of the nature of this activity during disease exists. In further work the contribution made to pattern and sensation of breathing from vagal C-fibres, which could not readily be investigated in this study, could be assessed in an animal model of emphysema in a larger species in which C-fibre activity can be recorded more easily.

Alterations in pattern of breathing and reflex activity have been found in this study which can be related to changes in lung receptor activity in the model of emphysema. Changes in lung architecture are probably responsible for these changes. In future work I think it is important to determine whether the changes in structure of the lungs of such a model, which are similar to those seen in human patients, produce changes in respiratory drive and if so to determine the origins of these changes.

A number of investigators have suggested that respiratory reflexes are involved in the patterns of breathing seen in a variety of lung injuries used to model naturally occurring disease, (Frankstein and Sergeeva, 1966), (Guz, Nobel, Eisele and Trenchard, 1969), (Armstrong, Luck and Martin, 1976), (Armstrong and Miller, 1980) and related them to control of breathing in disease. However none have:-

- a). Quantified the changes in amount and pattern of respiratory drive in an adequate model of human emphysema.
- b). Identified the pulmonary receptor groups from which such a change in drive might originate.

c). Addressed the fundamental question of whether sensation is required to stimulate breathing, and hence produce "pink and puffing" and "blue bloated" patients in which it is difficult to see evidence of respiratory drive.

An investigation to describe changes in respiratory drive caused by pulmonary emphysema and to identify their origin could be carried out in the following way. The measurement of pattern of breathing of rabbits before and after induction of emphysema would be made. Firstly, conscious breathing pattern would be measured in a whole body plethysmograph with the rabbits in their control state, this would then be repeated when emphysema had been induced in them. Anaesthetised pattern of breathing would also be recorded in the diseased rabbits. Comparisons of the differences between the conscious/anaesthetised and normal/diseased states would provide insight into the part played by sensation in stimulating breathing in emphysema.

Bulk and single-fibre phrenic activity would be recorded in control and emphysematous rabbits during eupnoea, while breathing is accelerated by carbon dioxide, and during reflexes generated by inflation and deflation of the lungs.

The progressive vagal block of first stretch receptors with sulphur dioxide, then all myelinated receptors by vagal cooling, and finally all receptors (by vagotomy) would identify the receptor type responsible for the changes in respiratory drive. Receptor sites could be then located.

The importance of using phrenic activity to outline the instantaneous change in respiratory drive is emphasised by the work of Bartoli, Cross, Guz, Huszczuk and Jefferies, (1975) and Cross, Jones and Guz, (1980), who demonstrated that "the pattern of vagally-mediated volume information during inspiration determines both shape and duration of phrenic motoneuron output." Thus continuous monitoring of respiratory drive, in the form of phrenic activity, is necessary to adequately describe changes in vagally modulated drive to breathe caused by disease.

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APPENDIX 1

DRUGS USED IN THE STUDY

1. Halothane (May and Baker UK.)
2. Crude Papain, No. p-3375 2.8 units/mg solid where 1 unit will hydrolyse 1.0 μ mole of Na-benzoyl-L-arginine ethyl ester (BAEE) per minute at pH 6.2 at 25⁰C, (Sigma, UK.). The Papain used was the crude substrate obtained from papaya latex , which was ground very finely in a pestle and mortar before making up in saline to administer to the animal.
3. Urethane, ethyl carbamate, (Sigma, UK.)
4. Xylocaine 2%, lignocaine hydrochloride, (Astra Pharmaceuticals LTD, UK.)
5. Heparin 5000 units/ml (CP Pharmaceuticals LTD, UK.)

APPENDIX 2

FIXATIVES AND REAGENTS USED FOR HISTOLOGY

All reagents used were of analar quality.

FIXATION FOR LIGHT MICROSCOPY

Formaldehyde 37-40% (Technical grade)

Sodium Chloride

EMBEDDING MEDIA AND REAGENTS

REAGENTS FOR GLYCOLMETHACRYLATE EMBEDDING

2-hydroxyethyl methacrylate

2-butoxyethanol

Benzoyl peroxide

Polyethylene glycol 400

N.N. dimethylaniline

FORMAL SALINE

10% formal saline was used to fix the whole lungs and it was made up as follows:-

100ml of technical grade formaldehyde

900ml of tap water

8.5g of NaCl

Marble chips to act as a buffer by neutralising any Formic acid produced.

The formal saline was stored in an airtight container until required. A fresh batch was made for each experiment.

APPENDIX 3

COMPUTER SCRIPT USED FOR ANALYSING DATA IN THE SPIKE2, (CED) SOFTWARE PACKAGE.

'BREATH ANALYSER VERSION 1.31

```
var time maxbrths breaths maxspiks strptr
var nospikes store[2000] nums[200] nums2[200] brtime[20]
```

```
maxbrths:=20
maxspiks:=100
strptr:=1
breaths:=0
analyse
```

```
end
```

```
proc setup
  off 3 4
  yrange 1 -1 1
  imeanfr 5
  draw 0 10
end
```

```
proc setviews;
  view 1; window 0 0 100 65;
  view 2; window 51 67 100 90;
  view 3; window 0 67 50 90;
  clear; view 1; draw
end
```

```
proc printnms
```

```
var loop ba bb bc bd
```



```

var ca cb cc cd ps$

i:=1
ps$:="          %3.0d-%7.5d %3.0d-%7.5d %3.0d-%7.5d %3.0d-%7.5d"
repeat
  ba:=nums[i] ; bb:=nums[i+1] ; bc:=nums[i+2] ; bd:=nums[i+3]
  ca:=nums2[i] ; cb:=nums2[i+1] ; cc:=nums2[i+2] ; cd:=nums2[i+3]
  print 2 ps$ ca ba cb bb cc bc cd bd
  i:=i+4
until i>nospikes
return
end

proc comdata

var lc ic

if breaths<>0
  for lc:=1 maxbrths-breaths
    print 3 ""
  next lc
  for lc:=1 maxspiks
    for ic:=0 breaths-1
      print -3 "%d," store[maxspiks*ic+lc]
    next ic
    print 3 "%d" 1
  next lc
  for lc:=1 maxspiks
    for ic:=0 breaths-1
      print -3 "%d," (store[maxspiks*ic+lc]/brtime[ic])*100
    next ic
    print 3 "%d" lc
  next lc
endif
return
end

```

proc analyse

```
var c1pos c2pos maxfreq minfreq spperbin aspperbn loop minvih
var maxvih minpih maxpih sampval meanfreq minspint
var maxspint minvth maxvth minpth maxpth timene
var printer integral hardcopy result
var anstat annum$ fibnum vagistat
var conapp condesc$ phase outfile$ oldfile$
var aa ab ac ad ae af ag ah ai
var aj ak al am an ao ap aq
var ps$ err comment$ anmode finish commit
var lcount pcnttime mc nc nlinec temp lasttime ps2$
var chn avval prevtime newtime
```

```
menu 0 "Receptor Analysis Program" 4
menu 1 1 "State of animal" 4 "Control" "Emphysema"
menu 1 2 "Animal number" 3 9
menu 1 3 "Fibre number" 2 0 10
menu 1 4 "State of Vagi" 4 "Intact" "Unilateral" "Bilateral"
anstat:=0 ; vagistat:=0 ;
menu 2 result anstat annum$ fibnum vagistat
```

```
hcursor 1
cursors 2
time:=0
query "is there a printer ?" "y or n then enter" "n" printer
loop:=0
outfile$=""
repeat
  loop:=loop+1
  err:=0
  repeat
    err:=1
    oldfile$:=outfile$
    conapp:=0 ; phase:=0
    menu 0 "Analysis Loop" 7
    menu 1 1 "Condition applied" 4 "Control" "Inflation" "Deflation" "CO2"
```

```

menu 1 2 "Condition description" 3 20
menu 1 3 "Comment" 3 3
menu 1 4 "Phase" 4 "Ti" "Te" "Sec1" "Sec2" "Sec3" "H.B."
menu 1 5 "Output filename" 3 12
menu 1 6 "Analysis mode" 4 "Fast" "Slow"
menu 1 7 "Finished" 4 "No" "Yes"
menu 2 result conapp condesc$ comment$ phase outfile$ anmode finish
if finish=1
  if vagistat<>0
    comdata
  endif
  return
endif
if oldfile$<>outfile$ ;
  file outfile$ err
  if err=2 ; message "This file exists already" ; endif
endif
until err=1
if printer=1.0
  if anstat=0 ; ps$="Control" ; else ; ps$="Emphysema" ; endif
  print 2 "          Outfile name          %s" outfile$
  print 2 "          State of animal        %s" ps$
  print 2 "          Animal number          %s" annum$
  print 2 "          Fibre number            %d" fibnum
  if vagistat=0 ; ps$="Intact" ; else ;
    if vagistat=1 ; ps$="Unilateral" ; else; ps$="Bilateral"
  endif
endif
  print 2 "          State of Vagi            %s" ps$
  if conapp=0 ; ps$="Control" ; endif
  if conapp=1 ; ps$="Inflation" ; endif
  if conapp=2 ; ps$="Deflation" ; endif
  if conapp=3 ; ps$="CO2" ; endif
  print 2 "          Condition applied        %s" ps$
  print 2 "          Condition description    %s" condesc$
  print 2 "          Comment                  %s" comment$
  if phase=0 ; ps$="Ti" ; endif

```

```

if phase=1 ; ps$="Te" ; endif
if phase=2 ; ps$="Sec1" ; endif
if phase=3 ; ps$="Sec2" ; endif
if phase=4 ; ps$="Sec3" ; endif
if phase=5 ; ps$="H.B." ; endif
print 2 ""
print 2 "          This run is %s" ps$
print 2 ""
endif

```

```

interact "Please set cursors, then hit return"
c1pos:=c1
c2pos:=c2
time:=c2pos-c1pos
print "time = %d" time
if printer=1.0
print 2 "          time          = %10.5d" time
endif

```

```

chn:=1
newtime:=c1 ; integral:=0 ;
if phase<2
repeat
prevtime:=newtime
nexttime chn prevtime newtime sampval
count chn prevtime newtime avval
avval:=abs(avval-HC)
integral:=integral+(avval*(newtime-prevtime))
until newtime>=c2
integral:=integral-(avval*(newtime-prevtime))
count chn prevtime c2 avval
avval:=abs(avval-HC)
integral:=integral+(avval*(c2-prevtime))
endif
print "integral of channel 1 = %d" integral
if anmode=1 ; interact ; endif
if printer=1.0

```

```

print 2 "          integral of ch 1          = %10.5d" integral
endif
if vagistat<>0
    count 5 c1pos c2pos nospikes
    minmax 5 c1 c2 minspint maxspint
    print "minimum spike int. = %d" minspint
    if anmode=1 ; interact ; endif
    if printer=1.0
print 2 "          minimum spike int.          = %10.5d" minspint
    endif
    print "maximum spike int. = %d" maxspint
    if anmode=1 ; interact ; endif
    if printer=1.0
print 2 "          maximum spike int.          = %10.5d" maxspint
    endif
    maxfreq:=1/minspint
    minfreq:=1/maxspint
    meanfreq:=nospikes/(c2pos-c1pos)
    print "maximum frequency = %d" maxfreq
    if anmode=1 ; interact ; endif
    if printer=1.0
print 2 "          maximum frequency          = %10.5d" maxfreq
    endif
    print "mean frequency = %d" meanfreq
    if anmode=1 ; interact ; endif
    if printer=1.0
print 2 "          mean frequency          = %10.5d" meanfreq
    endif
    print "minimum frequency = %d" minfreq
    if anmode=1 ; interact ; endif
    if printer=1.0
print 2 "          minimum frequency          = %10.5d" minfreq
    endif
    print "no. of spikes between cursors = %d" nospikes
    if anmode=1 ; interact ; endif
    if printer=1.0
print 2 "          no. of spikes between cursors = %10.5d" nospikes

```

```

endif
spperbin:=nospikes/(c2pos-c1pos)
aspperbn:=spperbin/100
print "average/bin = %d" aspperbn
if anmode=1 ; interact ; endif
if printer=1.0
print 2 "      average/bin      = %10.5d" aspperbn
endif
view 1; setinth 3 5 maxspint/0.001 0.001; process c1 c2
if anmode=1 ; interact ; endif
view 3
minmax 1 0 maxtime-1 minvih maxvih minpih maxpih
yrange 1 0 maxvih ; draw
print "minimum value in int. hist. = %d" minvih
if printer=1.0
print 2 "      minimum value in int. hist  = %10.5d" minvih
endif
print "maximum value in int. hist = %d" maxvih
if anmode=1 ; interact ; endif
if printer=1.0
print 2 "      maximum value in int. hist  = %10.5d" maxvih
endif
print "min.pos. in int. hist =%d" minpih
if anmode=1 ; interact ; endif
if printer=1.0
print 2 "      min.pos. in int. hist      = %10.5d" minpih
endif
print "max.pos. in int. hist. = %d" maxpih
if anmode=1 ; interact ; endif
if printer=1.0
print 2 "      max.pos. in int hist.      = %10.5d" maxpih
endif
view 1; setpsth 2 5 (c2pos-c1pos)*100 0.01 0;

process c1 c2
view 2
minmax 1 0 maxtime-1 minvth maxvth minpth maxpth

```

```

yrange 1 0 maxvth ; draw
print "minimum value in histogram = %d" minvth
if anmode=1 ; interact ; endif
if printer=1.0
print 2 "          minimum value in histogram  = %10.5d" minvth
endif
print "maximum value in histogram = %d" maxvth
if anmode=1 ; interact ; endif
if printer=1.0
print 2 "          maximum value in histogram  = %10.5d" maxvth
endif
print "pos. of min. value in histogram = %d" minpth
if anmode=1 ; interact ; endif
if printer=1.0
print 2 "          pos of min. value in histogr = %10.5d" minpth
endif
print "pos. of max. value in histogram = %d" maxpth
if anmode=1 ; interact ; endif
if printer=1.0
print 2 "          pos. of max. value in histogr = %10.5d" maxpth
endif
query "is a hard copy required?" "y or n then enter" "n" hardcopy
if hardcopy=1.0; scrndump; endif
view 1; draw
endif
if oldfile$<>outfile$
if vagistat<>0
comdata
endif
printto outfile$
breaths:=0
strptr:=1
endif
query "Commit data to file?" "y or n then enter" "y" commit
if commit=1.0
aa:=time ; ab:=nospikes
if phase=0 ; ps$="i" ; endif

```



```

if phase=1 ; ps$="e" ; endif
if phase=2 ; ps$="Se1" ; endif
if phase=3 ; ps$="Se2" ; endif
if phase=4 ; ps$="Se3" ; endif
if phase=5 ; ps$="HB" ; endif
print -3 "%c%s%c,%c%s%c," 34 comment$ 34 34 ps$ 34
if vagistat<>0
    ac:=minspint ; ad:=maxspint ;
    ae:=maxfreq ; af:=meanfreq ; ag:=minfreq ; ah:=aspperbn ;
    print -3 "%d,%d,%d,%d,%d,%d,%d,%d," aa ab ac ad ae af ag ah
    ai:=minvih ; aj:=maxvih ; ak:=minpih ; al:=maxpih ;
    am:=minvth ; an:=maxvth ; ao:=minpth ; ap:=maxpth ;
    aq:=integral ;
    print -3 "%d,%d,%d,%d,%d,%d,%d,%d" ai aj ak al am an ao
    print 3 "%d,%d" ap aq
    brtime[breaths]:=time
    breaths:=breaths+1
    timene:=clpos
    for lcount:=1 nospikes
        nexttime 5 timene timene
        store[strptr]:=timene-clpos
        strptr:=strptr+1
    next lcount
    for lcount:=1 maxspiks-nospikes
        store[strptr]:=0
        strptr:=strptr+1
    next lcount
else ;
    print 3 "%d,%d" aa integral
endif ;
endif
if printer=1.0
    if vagistat<>0
        for lcount:=1 nospikes+4
            nums[lcount]:=0
            nums2[lcount]:=lcount
        next lcount
    end
end

```

```

timene:=c1pos
lasttime:=c1pos
for lcount:=1 nospikes
    nexttime 5 timene timene
    nums[lcount]:=timene-lasttime
    lasttime:=timene
next lcount
print 2 ""
print 2 "        Unsorted Spike Intervals : "
print 2 ""
prntnms
for mc:=nospikes-1 1 -1
    for nc:=1 mc
        if nums[nc]>nums[nc+1]
            temp:=nums[nc]
            nums[nc]:=nums[nc+1]
            nums[nc+1]:=temp
            temp:=nums2[nc]
            nums2[nc]:=nums2[nc+1]
            nums2[nc+1]:=temp
        endif
    next nc
next mc
print 2 ""
print 2 "        Sorted Spike Intervals : "
print 2 ""
prntnms
print 2 ""
endif
if vagistat<>0 ; print -2 "%c" 12 ; else ;
    print 2 ""
    print 2 ""
    if loop=4
        print 2 "%c" 12
        loop:=0
    endif
endif
endif

```

```
endif  
until escape  
end
```

□

APPENDIX 4

Tables App.4-1 to App.4-6 show the activity of the three types of inspiratory firing receptors in the control and emphysematous rats during eupnoic breathing. Results shown for activity of each type of receptor during both inspiration and expiration.

During Ti Phase of breathing	Control Rats (n=60)	Emphysematous Rats (n=25)
Mean Peak Frequency in HZ	49.76 +/- 4.33	42.3 +/- 2.36
Mean minimum Frequency in HZ	25.06 +/- 1.30	23.5 +/- 1.99
Number of Action Potentials / second	22.2 +/- 1.47	21.51 +/- 1.6
Number of Action Potentials / phase	8.30 +/- 0.58	8.4 +/- 0.74

Table App.4-1: *Type I SAR receptor during inspiration in controls.*

During Te Phase of breathing	Control Rats (n=60)	Emphysematous Rats (n=25)
Mean Peak Frequency in HZ	11.55 +/- 2.23	7.44 +/- 2.50
Mean minimum Frequency in HZ	9.35 +/- 1.75	4.9 +/- 1.60
Number of Action Potentials / second	2.36 +/- 0.30	1.68 +/- 0.41
Number of Action Potentials / phase	1.08 +/- 0.15	0.96 +/- 0.2

Table App.4-2: *Type 1 SAR during expiration in controls.*

During Ti Phase of breathing	Control Rats (n=135)	Emphysematous Rats (n=200)
Mean Peak Frequency in HZ	89.77 +/- 1.68	86.02 +/- 1.64
Mean minimum Frequency in HZ	41.64 +/- 0.69	40.10 +/- 0.36
Number of Action Potentials / second	62.73 +/- 1.16	57.18 +/- 1.22
Number of Action Potentials / phase	24.72 +/- 0.79	20.94 +/- 0.64

Table App.4-3: *Type 2 SAR during inspiration in controls.*

During Te Phase of breathing	Control Rats (n=135)	Emphysematous Rats (n=200)
Mean Peak Frequency in HZ	67.57 +/- 2.24	70.19 +/- 9.72
Mean minimum Frequency in HZ	34.33 +/- 1.43	35.56 +/- 1.48
Number of Action Potentials / second	10.89 +/- 0.59	10.09 +/- 0.49
Number of Action Potentials / phase	4.96 +/- 0.25	4.55 +/- 0.21

Table App.4-4: *Type 2 SAR during expiration in controls.*

During Ti Phase of breathing	Control Rats (n=55)	Emphysematous Rats (n=65)
Mean Peak Frequency in HZ	114.46 +/- 4.82	125.07 +/- 13.85
Mean minimum Frequency in HZ	44.75 +/- 2.36	36.15 +/- 1.94
Number of Action Potentials / second	74.49 +/- 3.21	62.62 +/- 2.92
Number of Action Potentials / phase	30.16 +/- 1.22	23.0 +/-1.01

Table App.4-5: *Type 3 SAR during inspiration in controls.*

During Te Phase of breathing	Control Rats (n=11)	Emphysematous Rats (n=65)
Mean Peak Frequency in HZ	92.2 +/- 5.1	89.15 +/- 8.66
Mean minimum Frequency in HZ	17.32 +/- 1.92	14.17 +/- 1.09
Number of Action Potentials / second	32.00 +/- 2.26	29.81 +/- 1.64
Number of Action Potentials / phase	16.68 +/- 1.24	12.80 +/- 0.65

Table App.4-6: *Type 3 SAR during expiration in controls.*

The Type 1, 2 and 3 SARs were statistically significantly different from each other in terms of peak firing frequency, the number of action potentials firing per second and the number of action potentials firing per phase during both inspiration and expiration ($P<0.001$).

Receptor Type	Phase	n Breaths	Mean Peak Freq. in Hz	Mean Min Freq. in Hz	No. of Action Potentials per second	No. of Action Potentials per Phase
1	Ti	60	49.8 +/- 4.43	25.1 +/- 1.30	22.2 +/- 1.47	8.3 +/- 0.58
n=12	Te	60	11.6 +/-2.23	9.4 +/-1.75	2.4 +/-0.30	1.1 +/- 0.15
2	Ti	150	89.8 +/- 1.68	41.6 +/- 0.69	62.7 +/- 1.16	24.7 +/- 0.79
n=27	Te	150	67.6 +/- 2.24	34.3 +/- 1.43	10.9 +/- 0.59	5.0 +/- 0.25
3	Ti	45	114.5 +/- 4.82	44.8 +/- 2.36	74.5 +/- 3.21	30.2 +/- 1.22
n=11	Te	45	92.2 +/- 5.07	17.3 +/- 1.92	32.0 +/- 2.26	16.7 +/- 1.24
All SARs	Ti	250	86.1 +/- 1.98	39.0 +/- 0.79	57.4 +/- 1.69	22.4 +/- 0.71
n=50	Te	250	57.1 +/- 2.40	25.7 +/- 1.32	13.2 +/- 0.99	6.0 +/- 0.40
RARs	Ti	110	18.5 +/- 3.00	7.7 +/- 1.20	5.0 +/- 0.55	2.6 +/- 0.23
n=22	Te	110	87.5 +/- 6.10	18.1 +/- 1.08	28.8 +/- 1.25	13.3 +/- 0.55

Table App.4-7: Summarised results of discharge frequencies of receptor types from control rats during eupnoeic breathing.

APPENDIX 5

COMPARISON OF THE LUNG REFLEXES IN ANAESTHETISED RATS AND RABBITS.

INTRODUCTION.

A preliminary study into the pattern of breathing and lung reflex activity in rats and rabbits was carried out to compare the eupnoeic breathing pattern and reflex responses of the two species of animals to inflation and deflation pressures of 5 and 10cm of water pressure, Pirie and Davies, (1993).

METHOD.

The experimental protocol for the six rats used in this study was the same as for the main study described in chapter 2, however some slight modifications of this protocol was necessary for the rabbits.

EXPERIMENTAL PROTOCOL FOR RABBITS.

The rabbits were New Zealand Whites, supplied by B.K. Universal LTD UK. They ranged in weight 3.1-4.7 Kg, mean weight of the 6 rabbits used in this investigation was 3.68 ± 0.23 Kg. They were housed and fed in similar conditions to the rats. The rabbits were anaesthetised with an intravenous injection of 0.6ml/Kg sodium pentobarbitone, (Sagatal, May and Baker, UK.) administered into the right marginal ear vein. A catheter was tied into the left femoral vein and supplementary doses of urethane 25% solution were given to maintain surgical anaesthesia.

A plastic tracheal cannula of a suitable size and diameter (4mm inside 7mm outside diameter for the rabbits), whose internal tracheal diameter were 5mm was inserted and the rest of the procedure was as described for the rats in chapter 2.

Eupnoeic breathing pattern and responses of the rats and rabbits to lung inflation and deflation of the lungs to 5cm of water pressure were recorded. The method of recording and analysing data from the rats and the rabbits was as described in chapter 2 for the main study in rats.

RESULTS.

In both species of animals under urethane anaesthesia vagotomised breathing patterns were similar. The rats frequency of breathing was only 1.4 times faster than the rabbits. With the vagi intact Ti and Te shortened in both rats and rabbits increasing the breathing frequency in both species, see Table App 3-1. In the intact state breathing pattern of the two species was quite different as Ti and Te shortened more in the rats than they did in the rabbits. Ti was 2.6 times and 2.1 times shorter in the intact state than in the vagotomised in rats and rabbits respectively. While Te was 5.6 times and 1.3 times shorter in the intact state compared to the vagotomised state in rats and rabbits respectively. The influence of the vagi produced a slightly greater shortening of Ti and much greater shortening of Te of the rats compared to the rabbits. Therefore with the vagi intact the rats had a very much faster breathing rate than the rabbits, (3.7 times faster than the rabbits), see Figure App. 3-1.

	Rats	Rats	Rabbits	Rabbits
	Ti (s)	Te (s)	Ti (s)	Te (s)
Intact	0.22 +/- 0.01	0.25 +/- 0.01	0.55 +/- 0.01	1.18 +/- 0.04
Vagotomised	0.57 +/- 0.13	1.39 +/- 0.30	1.16 +/- 0.7	1.58 +/- 0.49

Table App. 3-1: *Eupnoeic pattern of breathing in rats and rabbits in the intact and vagotomised states showing the timing of inspiration and expiration in seconds.*

These results differ to those shown in the main results chapter since the data shown here is preliminary data from only six rats while the data in the main results chapter was from 16 rats.

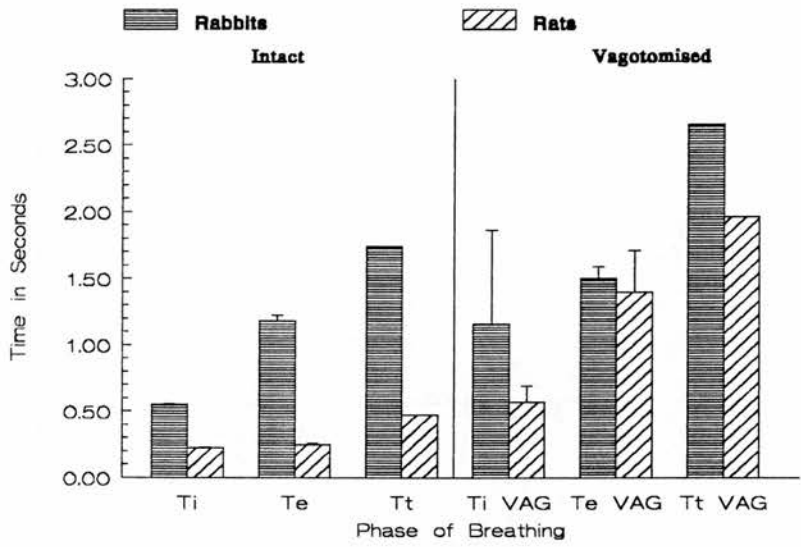


Figure App.3-1: *Eupnoeic intact and vagotomised pattern of breathing in rats and rabbits, shown in terms of duration of inspiration (Ti), expiration (Te) and total breath length (Tt).*

In the intact state the contribution to the total breath length from Ti and Te are about equal in the rats, 47% from Ti and 53% from Te. In the rabbits Te accounts for 58% of the total breath and Ti for 42%.

In the vagotomised state the contribution from each of the phases of breathing to the total breath duration was similar between the species. In rats the contribution of Tt from Ti and Te was 29% and 71% respectively while in the rabbits the contribution of Tt from Ti and Te was 26% and 74% respectively.

RESPONSE TO INFLATION PRESSURE.

INFLATION PRESSURE OF 5CM OF WATER.

The actual Hering-Breuer inflation reflex response to an intratracheal pressure of 5cm of water pressure was greater in rabbits than in rats. However when this was calculated as a ratio value of actual Hering-Breuer pause divided by the length of the

preceding eupnoeic T_e the ratio reflex response to inflation of 5cm H_2O was approximately equal in rats and rabbits see Figure App. 3-2.

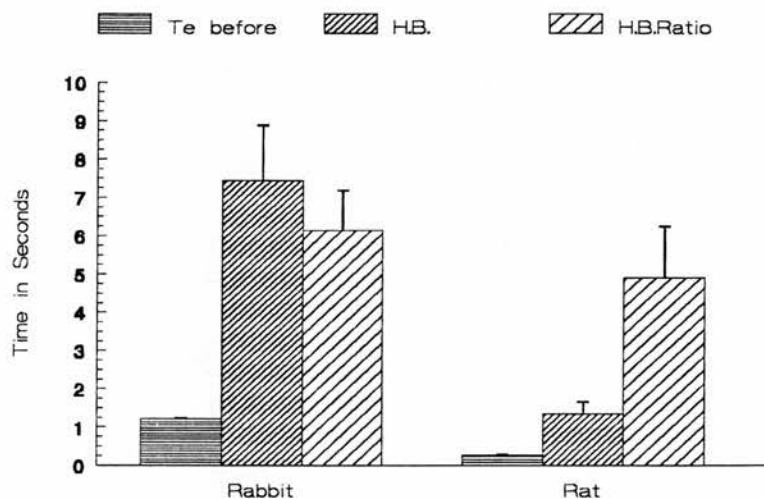


Figure App. 3-2: Response of rats and rabbits to an inflation pressure of 5cm of water. T_e before is the duration of the eupnoeic expiration immediately before inflation, HB -actual is the actual Hering-Breuer apnoea and HB ratio is the actual apnoea/ T_e immediately before inflation.

At an inflation pressure of 10cm of water pressure the actual Hering-Breuer inflation reflex was similar in both species. The Hering-Breuer inflation ratio was much greater in the rats than in the rabbits perhaps indicating the pressure of 10cm of water unsuitable for this small species.

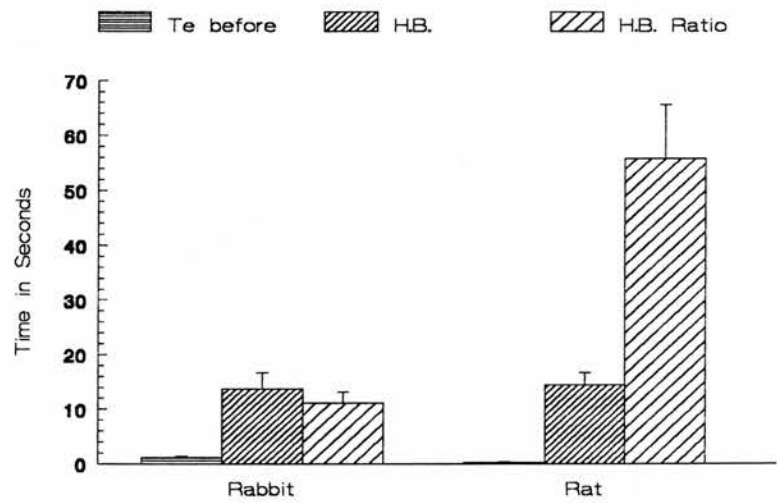


Figure App. 3-3: Response of rats and rabbits to an inflation pressure of 10 cm of water. *Te before* is the duration of the eupnoeic expiration immediately before inflation, *HB-actual* is the actual Hering-Breuer apnoea and *HB ratio* is the actual apnoea/ *Te* immediately before inflation.

RESPONSE TO DEFLATION PRESSURE.

Ti increased in both species in response to deflation of the lungs to 5cm of water pressure and Te decreased. Ti increased by about the same percentage in both of the species. In rabbits Ti increased by 30% and in rats Ti increased by 20%. The expiratory duration was a reduced much more in the rabbits than in the rats to values 30% control in rabbits and 80% control in rats see Figures App. 3-4 & 3-5.

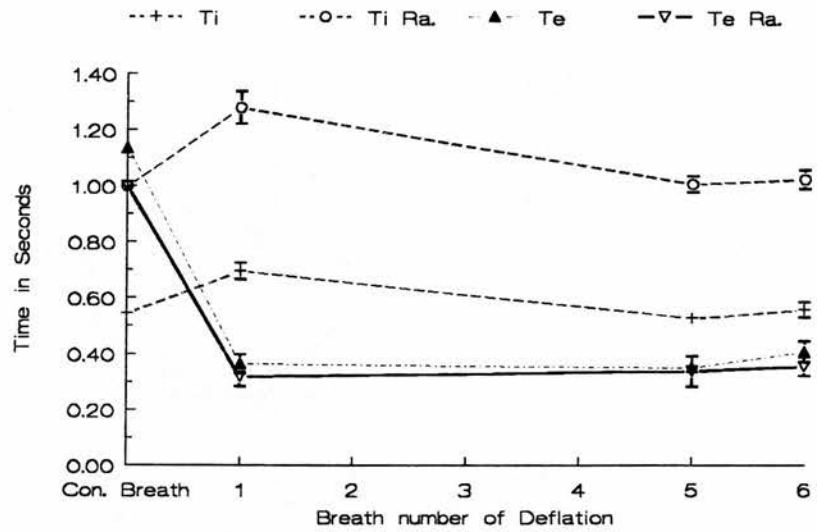


Figure App. 3-4: Response deflation pressure of 5cm of water in rabbits. Where TiRa and TeRa is the value of Ti and Te during deflation divided by their respective eupnoeic Ti or Te value before deflation.

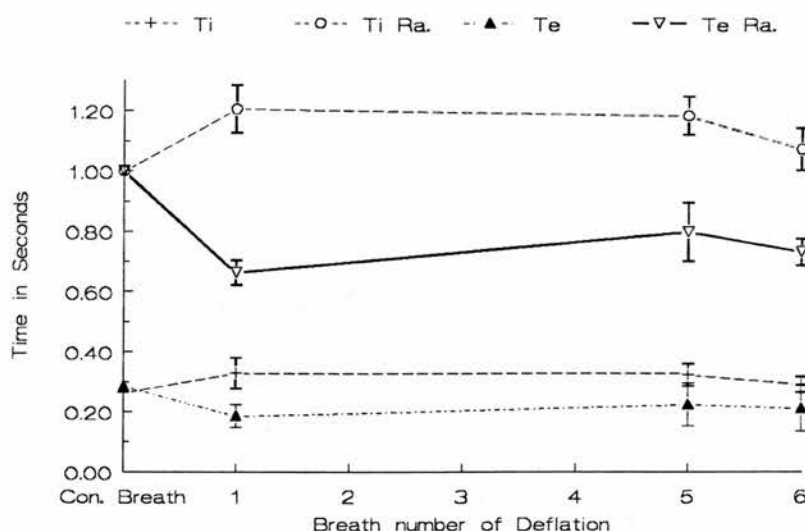


Figure App. 3-5: Response deflation pressure of 5cm of water in rats. Where $TiRa$ and $TeRa$ is the value of Ti and Te during deflation divided by their respective eupnoeic Ti or Te value before deflation.

DISCUSSION.

In the intact state with information from the vagus going to the respiratory centre the breathing pattern of the two species is quite different. The influence of the vagi had a much more profound effect on the pattern of breathing in rats than in the rabbits shown by the much greater shortening effect on Ti and Te in the rats than in the rabbits resulting in the rats having a significantly faster breathing rate in the intact state than the rabbits.

It would appear from these results that in the intact state in rats some factor may be shortening expiratory time that is not shortening expiratory time in rabbits, since when this factor is removed by vagotomy Te in rats increases while Te in rabbits virtually remains the same.

An explanation for the more profound increased Te observed in vagotomised rats could be the increased number of rapidly adapting receptors that we found in rats compared to in rabbits. We looked at 18 receptors in rats and rabbits and by my

criteria only 10% of the fibres were rapidly adapting in rabbits while 33% of them were rapidly adapting in rats. This could be the factor limiting T_e in the intact rats.

Emphasis on control of duration of breathing is often placed on inspiration, probably as this is the active part of breathing. However if designing a model for the control of breath length and therefore frequency of breathing it would seem appropriate to exert control over the part of breath which contributes most to its length. In this way a small percentage change in that phase of breathing would bring about a large change in the total time of a breath. So through control of the timing of expiration rather than inspiration greater control over the frequency of breathing could be made.

The slightly longer Hering-Breuer inflation apnoea ratio of the rabbits could be due to the greater proportion of slowly adapting stretch receptors in this species compared to the rats. The differences in reflex behaviour are probably due to the differing influence of vagal afferent receptors in the lungs of the two species

PUBLICATIONS

Publications arising from this thesis include the following:

Pirie, L., An inexpensive ultrasonic aerosol generator. 1993. J. Physiol 459: 303P.

Pirie, L., Davies, A. 1993. A comparison of the lung reflexes in anaesthetised rats and rabbits. J. Physiol. 459: 356P.

Pirie, L., Davies, A. 1995. Pulmonary receptors in the spontaneously breathing anaesthetised rat. J. Physiol. 483.P: 97P.

Davies, A., Pirie, L., Eyre-Todd, R.A. 1996. Adaptation of pulmonary receptors in the spontaneously breathing anaesthetized rat. Eur. Respir. J. 9: 1637-1642

Pulmonary receptors in the spontaneously breathing anaesthetized rat

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It has been suggested that the rate of adaption of pulmonary stretch receptors should be greater in species with high respiratory rates to maintain effective reflex control of breathing (Bartlett & St John, 1979). Our alternative suggestion is that a greater proportion of rapidly adapting receptors exists in more rapidly breathing species. This increases the overall rate of adaption. To test this hypothesis the activity of seventy-three pulmonary mechanoreceptors with afferent fibres in the left vagus nerve was studied in sixteen spontaneously breathing anaesthetized (6 ml kg^{-1} 25% Urethane, i.p.) rats, during eupnoea and sustained inflation of the lungs. Fifty-one receptors discharged mainly in inspiration, and were slowly adapting (PSRs). Twelve discharged exclusively during early inspiration. Thirty discharged throughout inspiration and in early expiration. Nine discharged throughout inspiration and expiration.

Twenty-two rapidly adapting receptors (RARs) were spontaneously active during eupnoea (peak frequency 87.52 ± 6.10 Hz, mean frequency 28.77 ± 1.25 Hz), discharged almost exclusively during expiration (2.59 ± 0.23 impulses in inspiration, 13.34 ± 0.55 impulses in expiration, 110 breaths) and, by definition, totally adapted in 0.25 s.

The patterns and total numbers of discharges for RARs were remarkably constant from breath to breath for individual receptors (e.g. 12.4 ± 0.4 impulses in five consecutive expirations).

The discharge frequencies of these receptors were comparable with those of other species (Widdicombe, 1954). The abundance of RARs was greater than in larger species (Roumy & Leitner, 1980).

The high proportion of RARs may represent an evolutionary advantage in neural control of the high frequency breathing of small mammals.

(Figures are means and standard errors of the mean).

Supported by the Norman Salvesen Emphysema Research Trust.

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An inexpensive ultrasonic aerosol generator

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Inhaled aerosols are used as experimental and therapeutic tools (Lourenco & Cotromanes, 1982). Ultrasonic aerosol generators offer many advantages over jet types. They produce a dense cloud, and therefore can deliver a high dose in a short time. The distribution of droplet size they produce, and therefore the site of deposition in the lungs, is narrower (Sterk *et al.* 1984). They do not require a supply of compressed air (Wright, 1958) and do not pressurize systems to which they are attached.

Their major disadvantages are that they are relatively expensive compared to other types, and usually require a relatively large charge of liquid to be aerosolized.

I will demonstrate an inexpensive ultrasonic aerosol generator, constructed from a commercial humidifier, which requires only small amounts of liquid to function.

Supported by the Norman Salvesen Emphysema Research Trust.

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A comparison of the lung reflexes in anaesthetized rats and rabbits

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We are using rats and rabbits to investigate changes in pattern of breathing, lung reflexes and lung receptor activity in papain-induced emphysema. While these respiratory variables have been documented for normal rabbits there is little information available about the behaviour of normal rats (Widdicombe, 1961; Young, 1980).

I have investigated rats and rabbits anaesthetized with Urethane (6 ml 25 % Urethane kg^{-1}). In both species vagotomized breathing patterns were similar. Inspiratory durations (t_I) were 1.16 ± 0.7 s (mean \pm S.E.M.) and 0.57 ± 0.13 s for rabbits ($n = 10$) and rats ($n = 20$) respectively, and expiratory durations (t_E) 1.58 ± 0.09 s and 1.39 ± 0.30 s respectively.

With vagi intact t_I and t_E were shortened to 0.55 ± 0.01 s and 1.18 ± 0.04 s respectively in rabbits and 0.22 ± 0.01 s and 0.25 ± 0.01 s in rats.

The Hering-Breuer inflation reflex in response to an intratracheal pressure of 0.5 kPa was approximately equal in rats and rabbits, but was longer in rats in response to a pressure of 1.0 kPa.

The response to a negative intratracheal pressure of 0.5 kPa was a reduction of t_E to 30 % control in rabbits and 80 % control in rats.

These differences in reflex behaviour are probably due to differences in afferent vagal activity from receptors in the lungs.

Supported by the Norman Salvesen Emphysema Research Trust.

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Adaptation of pulmonary receptors in the spontaneously breathing anaesthetized rat

A. Davies, L. Pirie, R.A. Eyre-Todd

Adaptation of pulmonary receptors in the spontaneously breathing anaesthetized rat
A. Davies, L. Pirie, R.A. Eyre-Todd. ©ERS Journals Ltd 1996.

ABSTRACT: It has been suggested that species with high breathing frequencies have pulmonary stretch receptors which adapt more rapidly than species with low breathing frequencies. This has proved not to be so. Our hypothesis is that this theory is in fact correct if modified so that overall rate of adaptation of afferent vagal activity, *i.e.* the sum of stretch and rapidly adapting receptors, is considered. A rapidly breathing species, such as the rat, would thus have a greater proportion of rapidly adapting receptors, than a more slowly breathing species.

To test this hypothesis, we measured the proportion of rapidly adapting pulmonary mechanoreceptors in spontaneously breathing rats for comparison with existing results from more slowly breathing species.

We found there to be one rapidly adapting receptor for every three slowly adapting receptors present. This measurement has not previously been made in spontaneously breathing rats. The ratio of rapidly to slowly adapting pulmonary receptors in the species sequence cat-rabbit-rat is the same as the ratio of their breathing frequencies (3:4:10).

We propose that the difference in proportion of slowly to rapidly adapting pulmonary receptors in different species may be related to their eupnoeic breathing frequency.

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BARTLETT and ST JOHN [1] made the important observation that adaptation of pulmonary mechanoreceptors imbues them with dynamic as well as static response characteristics. It would, thus, seem important to consider the degree of adaptation that takes place in a time interval similar to the animal's respiratory frequency. BARTLETT and ST JOHN [1] postulated that pulmonary stretch receptors (PSRs) of species with different eupnoeic frequencies would have different rates of adaptation to accurately signal lung conditions. This did not prove to be the case from their results. However, their observations were restricted to pulmonary stretch receptors.

Rapidly adapting receptors (RARs), sometimes called "irritant" or "deflation" receptors, are found in the lungs of many species [2]. It is possible that a change in the proportion of these receptors, relative to the number of PSRs, provides the different degree of adaptation required. The important concept of a link between receptor adaptation rate and frequency of eupnoeic breathing is linked to the role of PSRs and RARs in control of breathing. It is generally accepted that PSRs terminate inspiration and extend expiration. We have demonstrated [3] that RARs terminate expiration, and hence can profoundly affect breathing frequency. We have excluded C-fibre receptors from the present study because there is, as yet, insufficient quantitative description of their activity to enable between species comparison to be made.

Two recent publications [4, 5] have reported the activity of pulmonary receptors in anaesthetized, paralysed,

open- and closed-chested rats, ventilated by positive pressure. These authors report very little RAR activity. If this were true for intact spontaneously breathing rats, it would oppose the theory that small mammals with high respiratory rates have a higher proportion of rapidly adapting lung receptors.

We have used the rat as a model of respiratory control in human lung disease [6]. If the rat is to be a useful model in this context, it is important to know whether results obtained can be compared with those obtained in cats dogs and rabbits [3, 7, 8].

There is, as yet, insufficient evidence from a wide variety of species to give a categorical answer to the question of whether RAR have the same function in rats as in other species. However, drawing a parallel with PSRs, the Hering Breuer inflation reflex in all species, although varying in strength between species, is in all cases attributed to PSRs. Also, the way in which the number of receptors of a certain type in a species is determined needs to be considered and depends on the definition used. The overlap of the conduction velocities in fibres from PSRs and RARs tends to make categorization by this criterion difficult. However, these differences in definition only become important when considering subgroups of RARs and PSRs. In our experience with rabbits [3] and rats, the functional difference is unambiguous.

To determine whether the reported absence of RARs in rats was due to the nature of the preparation or a true species difference, and to measure the proportion of RARs

present, we recorded the activity of pulmonary receptors in closed-chested, spontaneously breathing rats during eupnoea, and during activity provoked by inflation of the lungs.

Methods

Animals and preparation

Fifteen barrier reared Sprague-Dawley rats, weighing 564 ± 21.4 g, were anaesthetized with an intraperitoneal injection of $1.5 \text{ g} \cdot \text{kg}^{-1}$ urethane as a 25% solution, supplemented as necessary *via* a catheter in the left femoral vein. A short tracheal cannula was inserted and airflow recorded by a Fleisch pneumotachograph head and Mercury CS5 differential pressure transducer. The left vagus nerve was cut high in the neck and the distal cut end placed in a copper tray filled with liquid paraffin. "Single fibre" preparations were made from strands of nerve that displayed respiratory rhythm when placed on a pair of silver wire electrodes. Carbon dioxide in the respired air was monitored by a Beckman L.B.1 gas analyser.

Experimental method

A period of eupnoeic breathing was recorded. The rat's lungs were then inflated four times with 0.5 and 1 kPa airway pressure. Three minutes separated the inflations, which were administered by the method of DAVIES and ROUMY [3]. This consisted of having a solenoid operated valve very rapidly connect the tracheal cannula from the atmosphere to a 50 L drum maintained at the required pressure. Inflation was synchronized with the peak of inspiration as detected by zero flow. Positive pressure was maintained until the rat took a spontaneous inspiration.

Recording

Electrical activity of the single fibre preparations was amplified by a high-gain RC amplifier (Neurolog), fed to an audio-amplifier and loudspeaker and recorded directly, and as transistor-transistor logic (TTL) pulses, together with the other physiological variables, by a TEAC XR-30 recorder.

Records were taken for four breaths before applying the step in pressure, and for 2–3 s after the first inspiratory effort.

Analysis

Analysis was undertaken "off-line" by digitizing the tape records *via* a Cambridge Electronics Design 1401 converter; and using a modified proprietary computer analysis program (Cambridge Electronics Design Spike 2).

Receptors were classified as slowly or rapidly adapting. Conduction velocity was initially used to differentiate between slowly and rapidly adapting receptors, but it soon became apparent that the difference in response to a step of inflation was sufficient to clearly differentiate between the two types. Adaptation was quantified using a form of KNOWLTON and LARRABEE [7] Adaptation Index, modified to provide criteria which enabled a

clear differentiation between slowly and rapidly adapting receptors, without the profound physiological interventions of paralysis or thoracotomy. Which would also have frustrated the objective of recording under eupnoeic conditions.

The number of action potentials in the third 0.25 s of lung inflation with a pressure of 1 kPa, was subtracted from the number in the first 0.25 s, and the result expressed as a percentage of the number of action potentials in the first 0.25 s of inflation. This expression of adaptation was termed "Adaptation Index", and clearly distinguished between slowly and rapidly adapting receptors. It also involved a time interval appropriate to the rat's breathing frequency. Rapidly adapting receptors were defined as having an index of 100%.

Occasionally, C-fibres, identified by their low conduction velocity, were isolated and discarded. Not all receptors, which could be clearly categorized manually as rapidly or slowly adapting, were suitable for computer analysis during inflation. The most common artifact defeating computer analysis was a second fibre discharging during inflation, which was not detected during recording.

Statistics

Statistical significance of difference between mean values, (shown as mean \pm SEM), calculated by Student's unpaired t-test was taken as a p-value less than 0.05. To ensure the maximum rigour, the mean values obtained for individual receptors were used for comparison.

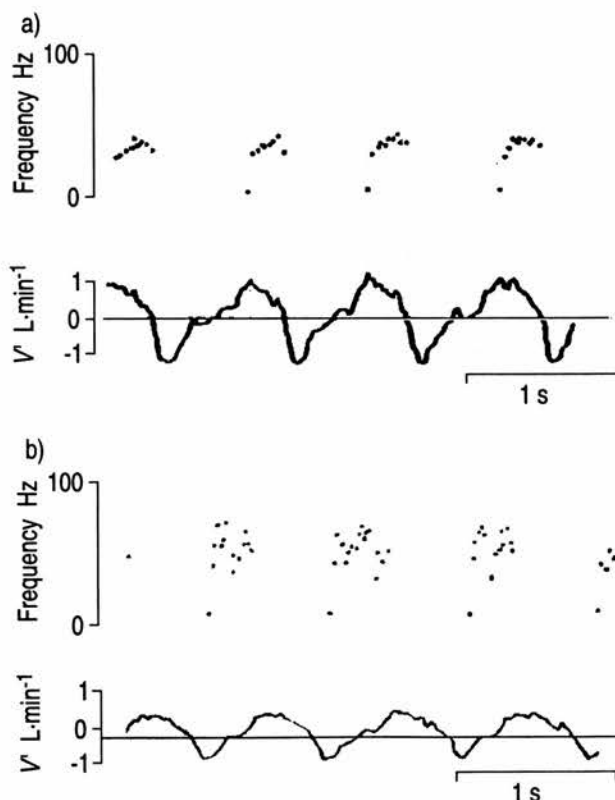


Fig. 1. — Computer record of respiratory airflow (expiration downward) and instantaneous frequency of discharge of: a) Type I PSR; b) expiratory RAR, in two rats. V : airflow; PSR: pulmonary stretch receptor; RAR: rapidly adapting receptor.

Results

Of the 85 receptors recorded, 12 were inadequate for later analysis. The 73 receptors characterized in this study could be divided into PSR (fig. 1a) and RAR (fig. 1b) on the criterion of the Adaptation Index described in the Methods section. All had the characteristics of receptors with myelinated fibres, when their discharge was inspected on an oscilloscope. Five fibres with discharge characteristics associated with unmyelinated fibres were abandoned.

In two rats, conduction velocity was measured in fibres firing in inspiration and seen to be slowly adapting (36.1 ± 7.1 ms⁻¹; $n=8$) and those firing in expiration which were rapidly adapting (14.6 ± 4.6 ms⁻¹; $n=4$).

The PSRs were divided into three types; and of these, two types, I and II, were very similar as described below. The mean adaptation index of PSR was $42.7 \pm 3.9\%$. The mean adaptation indices of the three individual types which will be described were: type I $58.0 \pm 8.8\%$; type II $42.6 \pm 1.8\%$; and type III $27.5 \pm 3.8\%$. Rapidly adapting receptors had an index of 100%.

Slowly adapting receptors (PSRs)

The receptors categorised as PSRs were further divided into three types on the basis of their discharge pattern during an eupnoeic respiratory cycle of mean tidal volume 2.83 ± 0.20 mL.

Type I (16% of all receptors). These 12 receptors discharged almost exclusively during mid and late inspiration as shown in figure 2. The small number of spikes (table 1a) occurring during expiration were restricted to the first 15% of expiratory duration (tE). Their peak, mean and minimum frequencies of discharge during eupnoea with a tidal volume (V_T) of 2.83 ± 0.20 mL and their frequencies on lung inflation (0.5 and 1.0 kPa) are shown in tables 1a and 2.

Type II (41% of all receptors). These 30 receptors were placed in a separate category from Type I because of their significantly higher discharge frequency (table 1a).

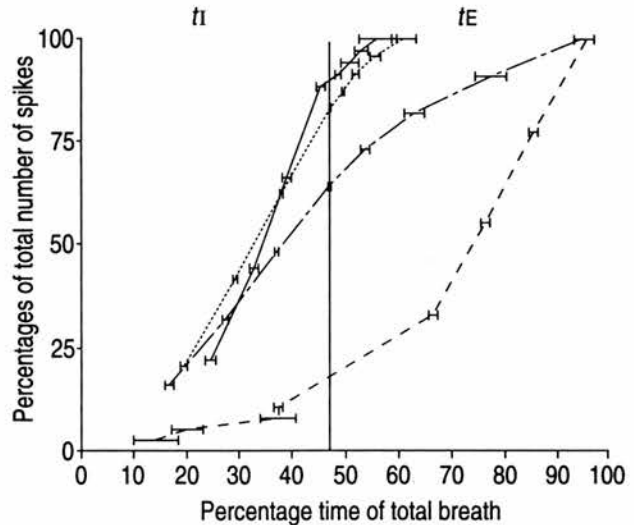


Fig. 2. — Distribution of the discharge of PSR Type I (12 receptors) PSR Type II (30 receptors), PSR Type III (9 receptors), and expiratory RARs (22) throughout a breath. The means \pm SEM of percentage of the total number of action potentials occurring at times through the respiratory cycle are shown. —: Type I;: Type II; - - - -: Type III; - . - .: RAR. tI : inspiratory duration; tE : expiratory duration. For further abbreviations see legend to figure 1.

During expiration, their discharge was restricted to the first quarter of tE . Thus, these receptors discharged over a somewhat greater range of the breathing cycle than Type I receptors. They were also placed in a separate category because of their lower adaptation index and significantly higher discharge frequency. Their response to eupnoea and sustained lung inflation is shown in tables 1a and 2.

Type III (12% of all receptors). These nine receptors differed more clearly from Type I and II than did Type I and II from each other. They discharged throughout inspiration and into expiration, as shown in figure 2. Their response to eupnoeic breathing and sustained lung inflation is shown in tables 1a and 2.

The only purpose of dividing the PSRs into Types I, II and III was to compare them with types reported by

Table 1. — Discharge characteristics of: a) pulmonary slowly adapting receptors and; b) rapidly adapting receptors

Receptor type	Phase	Receptors n	Peak frequency Hz	Mean frequency Hz	Minimum frequency Hz	Impulses in phase
a) Pulmonary slowly adapting receptors						
PSR I	tI	12	45.8 ± 5.2	20.4 ± 2.4	25.7 ± 2.4	8.3 ± 0.82
	tE	12	5.4 ± 3.7	1.79 ± 0.42	6.4 ± 2.5	1.08 ± 0.15
PSR II	tI	30	89.8 ± 3.7	62.7 ± 3.6	41.6 ± 1.3	24.7 ± 1.2
	tE	30	67.6 ± 7.5	10.9 ± 1.2	34.3 ± 2.3	5.0 ± 0.45
PSR III	tI	9	114.5 ± 10.1	74.5 ± 7.3	44.8 ± 4.7	30.2 ± 2.7
	tE	9	92.2 ± 8.0	32.0 ± 5.2	17.3 ± 4.2	16.7 ± 2.8
Mean all PSR	tI	51	86.1 ± 4.4	57.4 ± 3.8	40.0 ± 1.6	22.4 ± 1.4
	tE	51	57.1 ± 5.1	13.2 ± 2.0	25.7 ± 2.3	6.0 ± 0.99
b) Rapidly adapting receptors						
RAR	tI	22	18.5 ± 3.0	5.0 ± 1.3	7.7 ± 1.2	1.7 ± 0.48
	tE	22	87.5 ± 10.4	28.8 ± 3.0	18.1 ± 2.3	13.6 ± 1.4

Mean \pm SEM of peak and minimum frequency of discharge (Hz) of the four receptor types, measured from interspike interval. Mean frequency in this table = total spikes in the phase/duration of phase, and therefore can be less than minimum frequency, calculated from interspike interval. Frequencies of discharge of Types II and III receptors are significantly different ($p < 0.05$) from the mean of Type I receptors. For other criteria used to differentiate between receptor types see text. tI : inspiratory duration; tE : expiratory duration; PSR: pulmonary stretch receptor; RAR: rapidly adapting receptor.

Table 2. - Adaptation to inflation

Receptor type	Receptors n	Time from onset of inflation s	Impulses n	
			0.5 kPa	1.0 kPa
I	12	1st 0.25	15.8±1.2	19.2±2.3
		3rd 0.25	3.4±1.4	8.7±2.2
II	30	1st 0.25	25.5±2.1	31.6±4.1
		3rd 0.25	8.9±1.7	18.3±2.6
III	9	1st 0.25	31.0±2.1	31.7±2.2
		3rd 0.25	16.3±1.3	21.5±1.5

Mean±SEM of number of impulses (action potentials) in 1st and 3rd 0.25 s of lung inflations by 0.5 and 1.0 kPa for inspiratory receptor types I, II and III. Expiratory, rapidly adapting receptors, totally adapted within 0.25 s. The adaptation indices of Type I and Type II receptors were not significantly different. The adaptation index of Type III receptors was significantly different ($p<0.01$) from the other two types.

other workers (see Discussion). The pooled values of discharge properties of all the PSRs are, therefore, given in table 1a for comparison with those of the RARs, which was the main purpose of this investigation.

Rapidly adapting receptors (RARs)

These 22 receptors (30% of all receptors) discharged almost exclusively during expiration in eupnoeic breathing, as shown in figure 2 and table 1b.

Because "mean frequency" was calculated by dividing number of spikes by duration of inspiration (t_I) or expiration (t_E) it can, apparently paradoxically, be less than "minimum frequency", calculated from interspike interval.

Consistency of discharge. RARs have previously been described as irregular in their discharge. RARs in the rats used for these experiments discharged with a highly consistent pattern. Figure 3 shows the timing of 25, 50 and 100% of total discharge of two typical RARs over five consecutive breaths. Table 1b shows the discharge characteristics of 22 RARs during eupnoea.

Discussion

BARTLETT and ST JOHN [1] postulated that pulmonary mechanoreceptors of animals with different eupnoeic breathing frequencies would have different rates of adaptation. This did not prove to be the case from their results. However, their observations were restricted to PSRs. Rapidly adapting receptors, sometimes called "irritant" or "deflation" receptors, are found in the lungs of many species. It may be that a change in the proportion of RARs provides the different overall degree of adaptation required by different species [9]. Information concerning rapidly adapting receptors exists for a number of species [3, 10, 11]. No information has previously been available for rats. The association of adaptation rate with frequency of breathing receives support from our earlier findings [3] that the activity of RARs profoundly affects expiration, accelerating breathing.

In considering the control of pattern of breathing, it is important to consider the degree of receptor adaptation that takes place in a time interval approximately similar

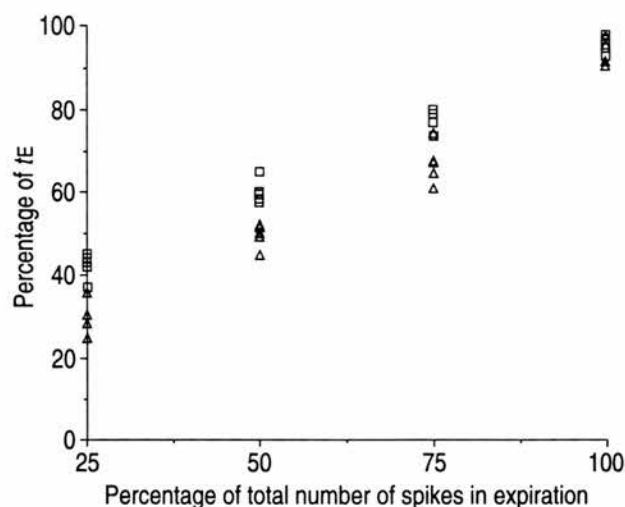


Fig. 3. - The regular nature of discharge of rapidly adapting receptors in rats, demonstrated by plotting percentage of duration of expiration (t_E) against percentage total number of spikes in expiration for five consecutive breaths for two typical rapidly adapting receptors (RARs). Δ : receptor 1; \square : receptor 2.

to the animal's respiratory cycle. The classical Adaptation Index of KNOWLTON and LARRABEE [7] involves a 2 s lung inflation. A rat might take three breaths in that time. WIDDICOMBE [10] points out that such an Adaptation Index "does not distinguish between adaptation rates of endings which cease firing within 1 s, so that stimuli well above threshold must be used and the endings must discharge for 2 s or more". To overcome these difficulties, we measured the degree of adaptation in 0.75 s of the application of a step stimulus.

Measured in the conventional open-chested preparation, rats have PSRs with adaptation indices similar to those of other species [4]. Our measure of adaptation is a more rigorous test of whether a receptor is rapidly or slowly adapting than conventional definitions. Recent publications [4, 5] have pointed out the paucity of information about the properties of pulmonary receptors in the rat and described discharge patterns mainly under conditions of respiratory paralysis and artificial ventilation.

We report here receptor activity measured without resort to the paralysis or thoracotomy of previous investigations, which would exclude recording under eupnoeic conditions. The difference between PSRs and RARs was very clear, and did not depend on our tentative division of PSRs into three types, undertaken for comparison with other published findings. Our findings are sufficiently similar to those reported previously to bear comparison, but differ in a number of important ways. Many differences may arise from the differences in transmural pressure found in the open- or closed-chested rats. That opening the chest affects receptor activity is clearly demonstrated in the publications by TUBON [5] and BERGREN and PETERSON [4]. In view of the profound differences between open- and closed-chested preparations, the properties of the PSRs in the report by BERGREN and PETERSON [4] and in our results are remarkably similar. We found 24% PSRs (16% of all receptors) to be exclusively inspiratory (Bergren and Peterson - 25%). Fifty nine of our PSRs (41% of all receptors) discharged throughout

inspiration and early expiration (Bergren and Peterson - 49%). Eighteen percent of our PSRs (12% of all receptors) discharged throughout the respiratory cycle.

As spontaneously breathing rats have respiratory frequencies of the order of 100 breaths·min⁻¹ [12] with virtually no expiratory pause, the "deflationary (D) slowly adapting receptors (SARs)" of BERGREN and PETERSON [4], which made up 18% of their SAR (PSR) population, and the "deflation sensitive receptors" of Tsubone [5], both of which were stimulated during the deflationary phase of their ventilating pump, have little equivalence to any of our receptors. One may speculate that these receptors would approximate more closely to one of the groups that we describe, if the rats from which they were recorded were breathing spontaneously rather than paralysed and ventilated by positive pressure.

Table 1 shows peak and minimum frequency derived from interspike intervals. Mean frequency is the number of action potentials in a phase of breathing (inspiration (I) or expiration (E)) divided by the duration of that phase, and can therefore be less than minimum frequency. Figure 2 shows the phase-spanning nature of the Type III receptors, and that Type I and RAR discharges are highly polarized into inspiration and expiration, respectively. The properties of Type I and Type II receptors are very similar. We have tentatively separated them into two types, mainly on the basis of adaptation rates and eupnoeic frequencies of discharge. WIDDICOMBE [10] comments "adaptation rate alone does not distinguish between different groups of pulmonary sense organs". It may well be that further investigation will not sustain this separation, which is not central to the thesis being tested by this study.

Because the imposed ventilatory cycles of paralysed rats used by other workers was so different from the pattern of spontaneous breathing of our rats, it is difficult to make comparisons of frequency of discharge. It can be said that the total number of impulses produced by Type I PSRs, in a respiratory cycle of inspiratory duration 0.39 ± 0.005 s and expiratory duration 0.43 ± 0.013 s ($n=60$ breaths; 12 rats) in our study, is of the same order of magnitude as all except the "mostly inspiratory" receptors reported by BERGREN and PETERSON [4] during a pump cycle of approximately 0.9 s; of which approximately 0.2 s was occupied by inflation. As most of the activity reported by these authors took place during inflation, there is an approximation to the rate of discharge that we report. SCHOENER and FRANKEL [13], who used a realistic frequency (2 Hz) to ventilate their paralysed rats, reported a mean discharge frequency of 96 ± 7 Hz for PSRs. This compares with our overall mean frequency for PSRs of 70.0 ± 1.7 impulses·s⁻¹ (table 1).

The algorithm that we used to measure adaptation rates of our receptors addressed the problems of matching the period investigated to a physiological breathing pattern, and the criticism by WIDDICOMBE [10] of the problem of attributing an adaptation index to receptors which silenced within 1 s of applying inflation. Our form of index also addresses the problem of using "peak frequency" in calculating adaptation index. Peak frequency, by definition, measures the time interval between only two action potentials.

Our longer, albeit very brief, interval provides a more representative sample of receptor activity on inflation.

Our adaptation index distinguished clearly between the three types of PSRs and RARs.

Pulmonary stretch receptors respond to the degree and rate of change of volume of the lungs [14], and have been categorized into those that saturate above 1 kPa transmural pressure and those with a more linear response. Some workers report [15, 16] that there is a continuum rather than discrete PSR types. Whilst this may be true for Type I and II PSRs found in our rats, these were very different in rate of adaptation and position of firing in the respiratory cycle from Type III PSRs or RARs (fig. 2 and tables 1 and 2). No attempt was made to identify the location of these receptors. Some may have been extrathoracic being active during expiratory flow [17].

In our rats, 30% of receptors active during spontaneous breathing were rapidly adapting. This is in direct contrast to BERGREN and PETERSON [4] who found only 7% of their receptors were rapidly adapting. Tsubone [5], on the other hand, using an open-chested paralysed preparation like BERGREN and PETERSON [4], found "irritant-like receptors", which discharged during both inflation and deflation. The only apparent difference between the methods of these two studies was the use by Bergren and Peterson of a 0.3–0.5 kPa end-expiratory pressure and the repeated use of exposures of 5–20 s to dimethyl-ether vapour to silence PSRs. Ether vapour stimulates RARs in guinea-pigs [18]. However, concentrations of 7.5–14.5% inhibits RARs [10]. Because of their more central and superficial position in the airways compared with PSRs [19], it is likely that the RARs in the study by BERGREN and PETERSON [4] received higher concentrations of the vapour used repeatedly to silence PSRs than the PSRs themselves. We cannot say whether such treatment permanently silences RARs, but it may explain the difference between the results of the latter study and our findings. It might also be that the use of end-expiratory pressure prevented atelectasis, which would have caused increased activity in the RARs of Tsubone's preparation compared to those of Bergren & Peterson.

There is a fundamental problem when comparing lung receptor activity of open-chested animals, ventilated by positive pressure, with the more physiologically normal spontaneously breathing animal. In the open-chested animal, the airways are expanded by a pressure which compresses the innermost epithelial layers against the underlying muscle. An important property of bronchial smooth muscle is that it becomes noncompliant at relatively low transpulmonary pressures (around 1 kPa [20]). Conversely, in normal, closed-chested, spontaneously breathing animals the innermost epithelial layers are placed in a state of tension by expansion of the lung. There are no data yet available as to whether these differences activate receptors in different ways; but it could be expected that RARs, being found in the innermost lining layers, would show greater differences than PSRs located within the muscle wall. It may be that the different patterns of RAR activity, in particular the presence of irregular activity during positive-pressure inflation ([8] for example) are, in part, a result of this effect.

In conclusion, we maintain that rapidly adapting receptors do exist in considerable numbers in rats and are active during spontaneous ventilation. They are present in the ratio of approximately 1 rapidly adapting recep-

tor to 3 pulmonary stretch receptors. This compares with ratios of 1:4 in the rabbit [11], and 1:10 in cats [10]. The breath durations of adults of these species is also in the ratio 3:4:10 [9]. This supports our suggestion (a modification of that of BARTLETT and ST JOHN [1]) that the respiratory frequency of a species is related to the overall adaptation rate of all its pulmonary receptors.

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